UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

ROSEMERI MOROKAWA

BIOGEOGRAPHY OF VIWITA CLADE AND PHYLOGENY OF WILLUGHBEIEAE (APOCYNACEAE, RAUVOLFIOIDEAE)

BIOGEOGRAFIA DO CLADO VIWITA E FILOGENIA DE WILLUGHBEIEAE (APOCYNACEAE, RAUVOLFIOIDEAE)

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Este exemplar corresponde à redação final da Tese defendida pela candidata

Rosemeri Morokawa

e aprovada pela Comissão Julgadora.

Thesis presented to the Institute of Biology the State University of Campinas in partial fulfillment of the requirements of the degree of Doctor in Plant Biology.

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas para obtenção do Título de Doutora em Biologia Vegetal.

Orientadora: Profa. Dra. Luiza Sumiko Kinoshita
Co-orientador: Prof. Dr. André Olmos Simões

CAMPINAS,
2014

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

Morokawa, Rosemeri, 1980-
M828b Biogeography of ViWiTa clade and phylogeny of Willughbeieae (Apocynaceae, Rauvolfioideae) / Rosemeri Morokawa. – Campinas, SP : [s.n.], 2014.

Orientador: Luiza Sumiko Kinoshita.
Coorientador: André Olmos Simões.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

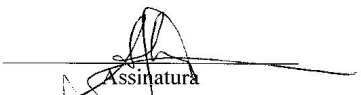
1. Taxonomia vegetal. 2. Filogenia. 3. Biogeografia. 4. Trópicos. I. Kinoshita, Luiza Sumiko,1947-. II. Simões, André Olmos. III. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Biogeografia do clado ViWiTa e filogenia de Willughbeieae (Apocynaceae, Rauvolfioideae)
Palavras-chave em inglês:
Plant taxonomists
Phylogeny
Biogeography
Tropics
Área de concentração: Biologia Vegetal
Titulação: Doutora em Biologia Vegetal
Banca examinadora:
Luiza Sumiko Kinoshita [Orientador]
Maria Ana Farinaccio
Marcelo Fragomeni Simon
Ingrid Koch
Maria do Carmo Estanislau do Amaral
Data de defesa: 28-08-2014
Programa de Pós-Graduação: Biologia Vegetal

Campinas, 28 de agosto de 2014

BANCA EXAMINADORA

Profa. Dra. Luiza Sumiko Kinoshita (orientadora) 
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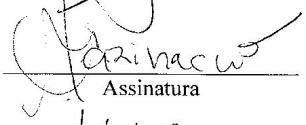
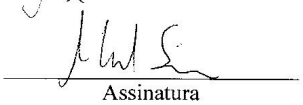
Profa. Dra. Maria Ana Farinaccio 
Assinatura

Dr. Marcelo Fragomeni Simon 
Assinatura

Profa. Dra. Ingrid Koch 
Assinatura

Profa. Dra. Maria Do Carmo Estanislau do Amaral 
Assinatura

Prof. Dr. Wellington Forster
Assinatura

Profa. Dra. Kikyo Yamamoto
Assinatura

Prof. Dr. João Semir
Assinatura

ABSTRACT

Apocynaceae is the second largest family of Gentianles, comprises 366 genera and ca. 5000 species and is divided in five subfamilies. An interesting group to study evolution and biogeography is ViWiTa clade, that comprises representatives of the three largest tribes (Vinceae, Willughbeieae, Tabernaemontaneae) within Rauvolfioideae. ViWiTa comprises 42 genera and 470 species distributed mainly in tropical regions of the world, with only one genus, *Vinca*, in temperate zone. The aims of the presente study are: to reconstruct a phylogenetic hypothesis of Willughbeieae, to test the monophyly of subtribes, to elucidate the phylogenetic relationships among its constituent genera, to identify morphological synapomorphies to the major clades (Chapter 1); to present a molecular dating to ViWiTa clade, to test biogeographical hypothesis about pantropical disjunction and test possible vicariant patterns and dispersal routes (Chapter 2). We performed phylogenetic analyses using chloroplast (*rpl*16, *rps*16, trnK, *trn*S-G and *mat*K) data from 97 specimens that belongs to 18 genera recognized to Willughbeieae. The percentagen of species sampled of each genus were above 60% in twelve genera, 50-59% in three genera and 30-49% in three genera. Molecular age estimates were calculated using a Bayesian approach based on 281 specimens belonging to 42 genera recognized in ViWiTa and five chloroplast markers. Ancestral areas were reconstructed using a maximum likelihood approach that implements the dispersal-extinction-cladogenesis model. Our study supports the monophyly of Willughbeieae and subtribes. We identified six possible synapomorphies to the

tribe: (1) liana habit; 2) style-head body apex undifferentiated; (3) style-head body base undifferentiated; (4) syncarpous ovary; and (5) parietal placentation; and (6) baccate fruits. The genera *Ancylobotris, Chamaeclitandra, Clitandra, Cylindropsis, Dyctiophleba, Orthopichonia, Pacouria, Saba* and *Vahadenia* were reduced to *Landolphia*. Necessary new combinations resulting from merging Landolphiinae genera into *Landolphia* were made. Dating and ancestral area analyses indicate that ViWiTa clade probably originated on early Paleocene in Australasia followed by migration via the Boretropics and subsequent colonizations to the Neotropics, Africa and Madagascar. Multiple long-distance dispersal events were inferred, such as from Africa to the Neotropics in Willughbeieae, from the Neotropics to Hawaii in Vinceae, and from Madagascar to Australasia in Tabernaemontaneae.

RESUMO

Apocynaceae é a segunda maior família de Gentianales com 366 gêneros e cerca de 5000 espécies, está dividida em cinco subfamílias. Um interessante grupo para estudar aspectos evolutivos e biogeográficos é o clado ViWiTa, que compreende as três maiores tribos (Vinceae, Willughbeieae, Tabernaemontaneae) de Rauvolfioideae, com 42 gêneros e 470 espécies distribuídas principalmente na região tropical do mundo, com exceção de *Vinca* em região temperada. Os objetivos do presente estudo são: reconstruir a hipótese filogenética de Willughbeieae, testar a monofilia das subtribos, elucidar as relações filogenéticas entre os gêneros e identificar sinapomorfias morfológicas para os clados maiores (Capítulo 1); realizar a datação molecular do clado ViWita, testar hipóteses biogeográficas acerca da disjunção pantropical e testar possíveis padrões vicariantes e rotas de dispersão (Capítulo 2). As análises filogenéticas foram realizadas usando cinco marcadores plastidiais (*rpl*16, *rps*16, trnK, *trn*S-G, *mat*K) para 97 espécimens pertencentes aos 18 gêneros reconhecidos em Willughbeieae. A porcentagem dos representantes amostrados para cada gênero foram acima de 60% em doze gêneros, 50-59% em três gêneros e 30-49% em três gêneros. As idades moleculares do clado ViWiTa foram estimadas a partir da hipótese filogenética de Willughbeieae, aliada as hipóteses filogenéticas existentes de Vinceae e Tabernaemontaneae, usando uma abordagem Bayesiana. A abordagem usada para reconstrução de áreas ancestrais foi a de Máxima Verossimilhança, que implementa o modelo de dispersão-extinção-cladogênise. Nossos resultados corroboram a monofilia da tribo e das subtribos: Lacmelleinae,

Leuconotidinae, Landolphiinae, Willughbeiinae. Seis sinapomorfias morfológicas para Willughbeieae são sugeridas: (1) hábito liana; (2) apice da cabeça do estilete indiferenciado; (3) base da cabeça do estilete indiferenciado; (4) ovário sincárpico; (5) placentação parietal; e (6) fruto baga. Os gêneros *Ancylobotris, Chamaeclitandra, Clitandra, Cylindropsis, Dyctiophleba, Orthopichonia, Pacouria, Saba* e *Vahadenia* foram sinonimizados em *Landolphia*. Análises de datação combinados com as análises de reconstrução de área ancestral sugerem uma provável origem do clado no início do Paleoceno na Australásia, seguida de migração via Boreotrópicos e subsequentes eventos de colonização para os neotrópicos, África e Madagascar. Multiplos eventos de dispersão à longa distância foram inferidos, como por exemplo da África para a região Neotropical em Willughbeieae, dos neotrópicos para o Havaí em Vinceae e de Madagascar para Australásia em Tabernaemontaneae.

Sumário

AGRADECIMENTOS

Ao Programa de Pós-graduação em Biologia Vegetal do Instituto de Biologia da Unicamp. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Conselho Nacional de Desenvolvimento Cientifíco e Tecnológico (CNPq) pela bolsa de doutorado e à Capes pela bolsa sanduíche. À Faepex/Unicamp pelo auxílio viagem para coleta de material. Ao Reflora CNPq/Fapesp pelo auxílio que permitiu a consulta de material nos herbários europeus, em especial Kew (Inglaterra) e Paris (França).

Ao apoio financeiro do projeto Fapesp "Estudos filogenéticos e evolutivos em Apocynaceae neotropical" coordenado pelo Prof. Dr. Olmos Simões, para a realização dos estudos moleculares.

Aos orientadores Profs. Drs. Luiza Sumiko Kinoshita e André Olmos Simões pelo apoio e ensinamentos.

À Universidade de Gotembugo, Suécia, por ter me aceito e pelo suporte oferecido e, ao supervisor Dr. Alexandre Antonelli, pelos ensinamentos e oportunidades.

Ao laboratório de Taxonomia Vegetal do Depto. de Biologia Vegetal do Instituto de Biologia da UNICAMP, pelas facilidades concedidas na realização deste trabalho. Aos laborátorios de Biologia Molecular da Escola de Artes, Ciências e Humanidades (EACH), USP e da Universidade de Gotemburgo, Suécia.

À todos os curadores e técnicos dos herbários visitados.

Aos membros da pré-banca e banca, Dra. Ingrid Koch, Dra. Maria Ana Farinaccio, Dra. Maria do Carmo Estanislau do Amaral e ao Dr. Marcelo Fragomeni Simon, pelas críticas e sugestões.

À Adrielli Matias, Ana Petri, André Scatigna, Amanda Shirleia, Atefeh Pirani, Bernard Pfeil, Brigitte Nyirambangutse, Camila Ferraz, Carlos Rodrigues, Claes Persson, Christini Caselli, Daiana Costa, Daniele Silvestro, Elisabet Sjökvist, Fabiana Firetti, Fernanda A. Carvalho, Fernanda Cabral, Fernanda Weiss, Filipe de Souza, Gislaine Rodrigues, Gustavo Heiden, Gustavo H. Shimizu, Hamid Moazzeni, João Afonso M. do Carmo, João Semir, Johan Dahlberg, Juan Francisco Morales, Juliana Bandeira, Juliana Philip, Karine B. de Souza, Kikyo Yamamoto, Marcela F. da Silveira, Marcelo M. Egea, Marcelo Moro, Maria Fernanda Z. Pombalino, Mary Endress, Mayá Schwade, Mayara Caddah, Mayara Morokawa, Meiriele Fogari, Michael Hopkins, Milena V. Martins, Milene Silvestrini, Nallarett Dávila, Nicoll Escobar, Norberto Anibal Maidana, Orachorn Mekkerdchoo, Patricia Luz Ribeiro, Rafaela Jorge, Renata S. Oliveira, Robin Foster, Rubens L. Coelho, Ruud Scharn, Suzana M. dos Santos Costa, Thomas Hasperg, Thyelle Cipriano, Tiago Barbosa, Tyana Wachter, Uiara Catharina, Vivian Alden, Wellington Forster, Ylva Heed e Zulqarnain Khattak.

INTRODUÇÃO GERAL

Apocynaceae

Apocynaceae *sensu lato,* a segunda família em número de espécies da ordem Gentianales, é uma das dez maiores de angiospermas e compreende cerca de 366 gêneros (Endress *et al.* 2014) e cerca de 5000 espécies (Meve 2002; Endress 2004). A família encontra-se amplamente distribuída nas regiões tropicais e subtropicais, com alguns poucos gêneros (*Apocynum* L.*, Nerium* L.*, Vinca* L. e *Vincentoxicum* Wolf, entre outros) presentes nas regiões temperadas (Mabberley 1997; Endress & Bruyns 2000; Rapini *et al.* 2003).

Os representantes de Apocynaceae são conhecidos no mundo por suas belas flores e o uso como plantas ornamentais, como por exemplo, *Nerium oleander* L. e espécies de *Allamanda* L., *Catharanthus* G. Don, *Hoya* R. BR., *Mandevilla* Lindl., *Plumeria* L., *Stapelia* L. Nos neotrópicos, algumas espécies de *Aspidosperma* Mart. & Zucc. fornecem madeira de alta qualidade utilizada na costrução de móveis. Diversas espécies da família possuem importantes compostos químicos para a medicina, sobretudo no látex que é usado por diversas comunidades tradicionais como plantas medicinais. Alguns frutos são comestíveis, principalmente nas espécies da tribo Willughbeieae (e.g., *Couma rigida* Müll. Arg., *Hancornia speciosa* Gomes, *Landolphia owariensis* P. Beauv., *Willughbeia edulis* Roxb.).

Endress & Bruyns (2000) propuseram a primeira classificação de Apocynaceae *s.l.*, baseando-se no conjunto de evidências morfo-anatômicas, químicas, cariológicas e macromoleculares disponíveis até então. Cinco

subfamílias foram reconhecidas por estes autores: Rauvolfioideae e Apocynoideae, correspondendo às Apocynaceae *s.str.*; Asclepiadoideae e Secamonoideae, correspondendo às tradicionais Asclepiadaceae; e Periplocoideae, correspondendo à Periplocaceae. Entretanto, esta divisão em subfamílias ainda é discutível, pois não reflete uma classificação filogenética, uma vez que diversos estudos indicam a parafilia de Rauvolfioideae e Apocynoideae (Rapini *et al.* 2003; Ionta & Judd 2007; Lahaye *et al.* 2007; Livshultz *et al.* 2007; Simões *et al.* 2007; Livshultz 2010), e existe também a incerteza no posicionamento de Periplocoideae dentro da família. Endress & Bruyns (2000) também propuseram uma divisão em 17 tribos, classificação esta que teve boa aceitação pelos pesquisadores e que foi atualizada para Rauvolfioideae (Simões *et al.* 2007; Endress *et al.* 2007; Simões *et al.* 2010), Apocynoideae (Sennblad & Bremer 2002; Simões *et al.* 2004; Livshultz *et al.* 2007; Livshultz 2010), Secamonoideae (Lahaye *et al.* 2005, 2007) e Asclepiadoideae (Liede *et al.* 2002; Rapini *et al.* 2003, 2006; Liede-Schumann *et al.* 2005; Wanntorp & Foster 2007; Hechem *et al.* 2011). O recente trabalho de Endress *et al.* (2014) atualiza o número de tribos para 25 e 49 subtribos em Apocynaceae.

Rauvolfioideae e o clado ViWiTA

A subfamília Rauvolfioideae consiste de plantas com flores sem fusão pós-genital dos estames com a cabeça do estilete, sementes não comosas e uma grande diversidade de frutos. Nas classificações propostas por Schumann (1895), Pichon (1948) e Leeuwenberg (1994), o tipo de fruto e semente eram importantes na delimitação de tribos em Rauvolfioideae, devido à diversidade

morfológica. No entanto, estudos filogenéticos moleculares sugerem que os caracteres de frutos e sementes são homoplásticos e que as classificações baseadas nestes caracteres tem formado grupos não monofiléticos (Potgieter & Albert 2001, Simões *et al*. 2007).

Desde a classificação de Endress & Bruyns (2000), somente uma nova tribo, Aspidospermaeae, foi reconhecida em Rauvolfioideae (Simões *et al.* 2007), mas muitos gêneros tiveram sua posição alterada.

Dentre as tribos identificadas como monofiléticas por Simões *et al*. (2007, Fig. 1, pag. 30), podemos destacar o clado formado por Vinceae, Willughbeieae e Tabernaemontaneae (ViWiTa). O clado ViWiTa reune as três maiores tribos de Rauvolfioideae, com 470 espécies distribuídas nos neotrópicos e paleotrópicos, esta diversidade de espécies e padrões de distribuição faz deste clado um grupo modelo para estudos biogeográficos.

Tabernaemontaneae possui 15 gêneros e cerca de 180 espécies, as quais são caracterizadas pelas flores com anteras lignificadas, cabeça do estilete com ápice lobado, frutos bacáceos ou foliculares e sementes geralmente ariladas (Simões *et al.* 2010). Alguns gêneros (*Calocrater* K. Schum. & Prantl, *Carvalhoa* K. Schum. & Prantl, *Crioceras* Pierre e *Schizozygia* Baill.) são encontrados somente na África, enquanto outros (*Ambelania* Aubl.*, Macoubea* Aubl.*, Molongum* Pichon*, Mucoa* Zarucchi*, Neocouma* Pierre*, Rhigospira* Miers*, Spongiosperma* Zarucchi) são restritos aos neotrópicos. Somente um gênero possui uma ampla distribuição nos trópicos, *Tabernaemontana* L.*,* o maior gênero da tribo com cerca de 110 espécies ocorrentes na região Neotropical, África continental, Madagascar, sudeste da Ásia, Nova Caledônia e Austrália.

Voacanga Thouars, por outro lado, tem 12 espécies distribuídas na África tropical, Madagascar, sudeste da Ásia, Filipinas e Nova Caledônia, mas está ausente nos neotrópicos.

Vinceae é uma tribo com nove gêneros e cerca de 160 espécies. Oito gêneros encontram-se distribuídos na região tropical e um, *Vinca,* é exclusivo de regiões temperadas da Eurasia (Endress & Bruyns 2000). Em sua circunscrição atual (Endress *et al.* 2007, Simões *et al.* 2007), a tribo pode ser caracterizada pela seguinte combinação de caracteres morfológicos: folhas opostas ou verticiladas, flores com anteras não lignificadas, e cabeça do estilete com anel basal membranáceo e frutos geralmente drupáceos com uma a poucas sementes. O gênero mais diversificado da tribo é *Rauvolfia* L., com cerca de 60 espécies; destas, pouco mais da metade encontra-se distribuída nos neotrópicos (Koch 2002), enquanto as espécies paleotropicais estão distribuídas principalmente na África continental, Madagascar, Havaí, Nova Caledônia, Ásia continental e ilhas da Oceania (Boiteau *et al.* 1976; van Dilst & Leeuwenberg 1991; Hendrian & Middleton 1999).

De acordo com Simões *et al.* (2007), a tribo Willughbeieae é constituída por 18 gêneros e cerca de 150 espécies; destes, cinco são exclusivamente neotropicais (*Couma* Aubl., *Hancornia* Gomes, *Lacmellea* H. Karst., *Pacouria* Aubl., *Parahancornia* Ducke) e 13 são exclusivamente paleotropicais (*Ancylobotrys* Pierre, *Bousigonia* Pierre, *Chamaeclitandra* (Stapf.) Pichon, *Clitandra* Benth., *Cyclocotyla* Stapf, *Cylindropsis* Pierre, *Dictyophleba* Pierre, *Landolphia* P. Beauv., *Leuconotis* Jack, *Ortopichonia* H. Huber, *Saba* (Pichon) Pichon, *Vahadenia* Stapf, *Willughbeia* Roxb.). As espécies paleotropicais são

lianas, sempre lenhosas, raro arbustos, com as inflorescências por vezes portando gavinhas, enquanto as espécies neotropicais são árvores de pequeno a grande porte, raro arbustivas, com inflorescências nunca modificadas em gavinhas, com exceção de *Pacouria*.

Pacouria é uma liana neotropical com inflorescências transformadas em gavinhas morfologicamente similar às espécies africanas de *Landolphia*. Por esse motivo, alguns autores (e.g. Monachino 1945) consideram *Pacouria* como sinônimo de *Landolphia*.

Cenários Biogeográficos

A ampla distribuição das espécies de Tabernaemontaneae, Vinceae e Willughbeieae na região tropical permitem a análise de diversos cenários biogeográficos, tanto em nível global quanto local, para explicar sua distribuição atual. Abaixo estão listados alguns dos cenários que serão testados:

I) Quebra Gonduânica: Gonduana era um supercontinente no hemisfério sul composto das atuais regiões da América do Sul, África, Antártica, Índia, Madagascar, Nova Guiné, Austrália, Nova Zelândia e Nova Caledônia. Neste cenário, a quebra do supercontinente Gonduânico por meio da separação das placas continentais originou a disjunção trans-tropical em diversos grupos, no qual as linhagens derivadas isoladas de um mesmo ancestral evoluíram independentemente. O cenário Gonduânico tem sido amplamente assumido para explicar a radiação das angiospermas durante o Cretáceo superior e as similaridades das floras tropicais da América do Sul e África (*e.g.* Raven & Axelrod 1974; Gentry 1982; Bumham & Graham 1999). Existe evidência fóssil da

origem Gonduânica de algumas famílias de plantas, tais como Arecaceae (Moore 1973) e Annonaceae (Schatz & Thomas 1993). No entanto, essa hipótese é problemática porque muitos grupos de angiospermas se originaram e diversificaram bem depois da conexão de terra existente há 105-95 Ma entre América do Sul e África (McLoughlin 2001). Essa hipótese é também pouco provável para explicar os padrões biogeográficos em ViWiTa, pois estudos sugerem que Apocynaceae surgiu por volta de 53-45 Ma no Eoceno (Magallón *et al*. 1999, Wikström *et al.* 2001).

II) Flora Boreotropical: do Paleoceno ao Eoceno (60-30 Ma), o clima global atingiu uma dais maiores temperaturas da história do planeta, alcançando ao máximo uma temperatura média global de aproximadamente 12°C maior que a atual (Zachos *et al.* 2008). Este período de aquecimento global proporcionou a troca da vegetação temperada a subtropical de toda o sul da Laurásia no hemisfério norte por uma vegetação tropical, referida por Wolfe (1975, 1977) como a "flora Boreotropical". Durante este período, a flora Boreotropical poderia ter se expandido rapidamente em sentido longitudinal sobre o hemisfério por duas conexões de terra. A primeira, conhecida como ponte de terra de Bering ou Beríngia, que era uma conexão entre América do Norte e Laurásia pela ponte de terra Alasca-Sibéria, em altas latitudes (entre 69°N e 75°N). O outro, chamado de ponte de terra do Atlântico Norte, era uma ligação entre Europa e América do Norte por duas rotas possíveis, no norte "DeGeer", do norte da Escandinávia ao norte da Groenlândia, e uma ao sul "Thulean", do norte da Escócia para o sul da Groenlândia (McKenna 1983, Tiffney 1985).

Destas duas rotas possíveis, Beríngia é a menos provável para ter funcionado como uma possível rota de troca de linhagens tropicais da América à Ásia, devido às suas condições climáticas e limitada exposição de luz (Tiffney 1985). Segundo Brikiatis (2014), a rota de Bering funcionou em dois períodos há 65 Ma, coincidindo com a rota De Geer, e há ca. 58 Ma, já a rota Thulean tornou-se estabelecida bem após a rota De Geer em pelo menos dois episódios ca. 57 Ma e 56 Ma.

A disjunção pantropical de alguns grupos de Angiospermas podem ser explicados pela rota de migração Boreotropical e muitos artigos sugerem que o corredor Atlântico Norte poderia explicar a presente distribuição de grupos tropicais nas regiões neotropicais e paleotropicais, e.g. Fabaceae (Lavin *et al.* 2000); Styracaceae (Fritsch 2001); Melastomataceae (Renner *et al.* 2001); Malpighiaceae (Davis *et al.* 2002a, b); Cornaceae (Xiang & Thomas 2008); Rubiaceae (Antonelli *et al.* 2009); *Guatteria*, Annonaceae (Erkens *et al*. 2009); Urophylleae, Rubiaceae (Smedmark *et al*. 2010); Myrsinaceae (Manns & Anderberg 2011) e *Cissus*, Vitaceae (Liu *et al*. 2013). A baixa representação ou mesmo a ausência atual desses grupos no Hemisfério Norte pode ser explicada pela diminuição da temperatura e o aumento na sazonalidade que ocorreu durante o Eoceno superior e na transição do Eoceno-Oligoceno. Este processo resultou na contração da flora Boreotropical e consequentemente na expansão geográfica da vegetação decídua na América do norte e Eurásia. Subsequentemente, a flora Boreotropical remanescente seguiu três caminhos possíveis: 1) retração em direção ao sul nas áreas de climas quentes que

correspondem à zona tropical atual; 2) confinamento a pequenas áreas, em refúgios; 3) extinção local (Tiffey 1985).

III) Dispersão à Longa-Distância: uma das questões mais discutidas na biogeografia tem sido o papel da dispersão à longa distancia como significante explicação para a distribuição atual. Com a proposta da vicariância como principal mecanismo de disjunção geográfica de linhagens feita pelo "*American Museum Group*" (Platnick & Nelson 1978; Nelson & Platnick 1981; Wiley 1981, 1988), a dispersão tornou-se um mecanismo secundário na explicação destas disjunções. Entretanto, com o avanço da sistemática molecular e a datação molecular de árvores filogenéticas, este ponto de vista não é sustentado por estudos recentes (Givnish *et al.* 2004; Queiroz 2005; Guzmán & Vargas 2009), os quais sugerem que disjunções em diversos grupos de plantas podem parcialmente ser explicadas em termos de eventos de dispersão a longa-distância, especialmente por eventos de dispersão transoceânica. Atualmente, a maioria das distribuições podem ser explicadas por uma combinação de eventos de dispersão e vicariância (Christenhusz & Chase 2013). Como por exemplo, no estudo de Michalak *et al*. (2010) com a pequena família pantropical, Hernandiaceae (Laurales), a primeira divergencia na família entre linhagens predominantemente Africa+Madagascar+Malesia e Africa+Neotropical pode ter acontecido há 122 (134-110) Ma, podendo ser congruente com a quebra da Gonduana Oeste e com posteriores dispersões transoceânicas durante o Oligoceno e Mioceno. As disjunções entre linhagens paleotropicais e neotropicais do clado ViWiTa, ou ao menos algumas delas, poderiam consequentemente ser explicadas por eventos de dispersão

transoceânica, como por ex., a colonização das ilhas Havaianas por espécies neotropicais e a colonização dos neotrópicos a partir de ancestrais africanos.

Os modelos acima descritos não são necessariamente excludentes, sendo que dois ou mais destes podem ter ocorrido simultaneamente, ou sequencialmente, durante a história evolutiva de um grupo. A determinação da sequência de eventos de cladogênese, somada à estimativa do tempo absoluto da origem do clado ViWiTa, permitirá fazer inferências sobre cada modelo e testar sua adequabilidade.

JUSTIFICATIVA

O recente acúmulo de estudos filogenéticos em Apocynaceae *s.l.* nos mais diferentes níveis taxonômicos trouxe avanços significativos na sistemática da família, porém são poucos os estudos biogeográficos moleculares publicados até o momento (Rapini *et al.* 2007).

O clado formado pelas tribos Vinceae, Tabernaemontaneae e Willughbeieae é uma excelente escolha para estudos biogeográficos e para compreender os padrões e processsos de diversificação na região tropical.

A distribuição pantropical das três tribos permite testar cenários biogeográficos globais, que expliquem as disjunções entre linhagens neotropicais e paleotropicais. Este trabalho vem a contribuir não somente com o entendimento de padrões biogeográficos em Apocynaceae, mas também fornecerá novos elementos para compreender estes padrões em angiospermas, principalmente em grupos pantropicais.

As tribos Tabernaemontaneae (Simões *et al.* 2010) e Vinceae (Simões *et al.*, in prep.) já foram estudadas e possuem hipóteses filogenéticas conhecidas. Já a tribo Willhugbeieae foi escolhida para estudo no presente trabalho.

OBJETIVOS

Esta tese é composta de dois capítulos, apresentados de acordo com os periódicos em que serão submetidos, com os seguintes objetivos:

(1) Inferir a filogenia da tribo Willhugbeieae com base em caracteres moleculares visando testar a monofilia das subtribos e dos gêneros na tribo (Capítulo 1);

(2) Produzir uma filogenia datada para o clado ViWiTa e testar hipóteses biogeográficas sobre a origem da distribuição pantropical desse clado (Capítulo 2).

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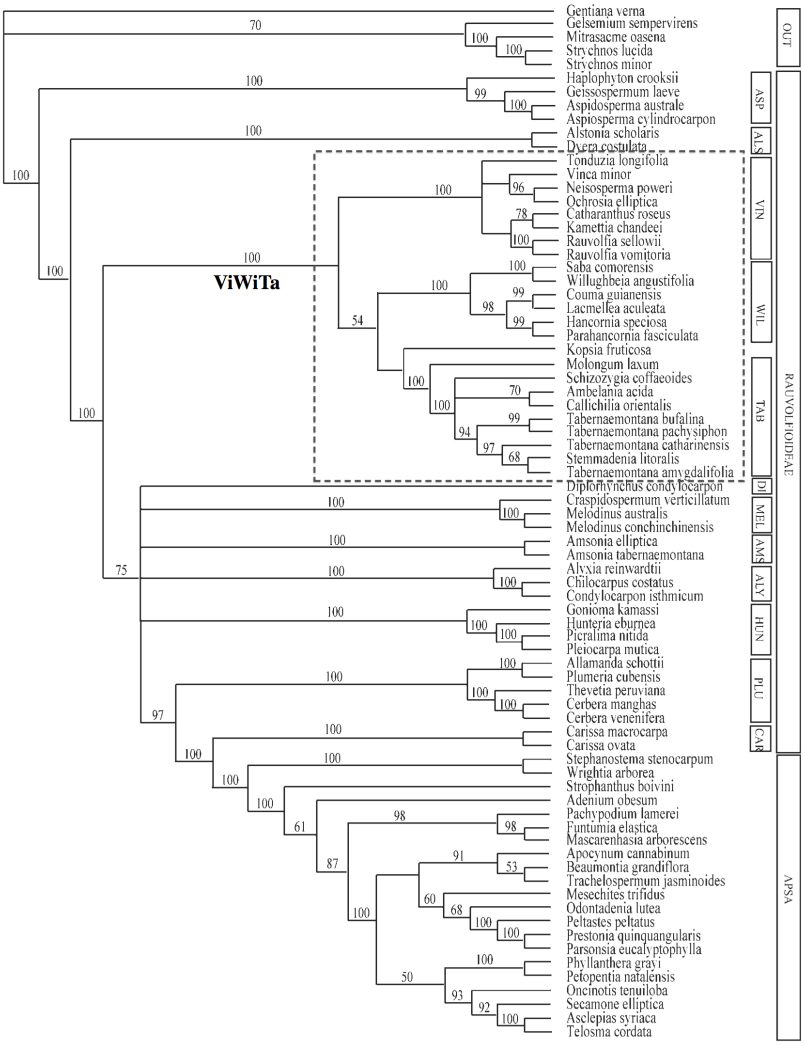

Fig. 1. Filogenia molecular de Rauvolfioideae com o clado ViWiTa em evidência. Os clados identificados são abreviados da seguinte forma: OUT = Outgroup; ASP = Aspidospermeae clade; ALS = Alstonieae clade; VIN = Vinceae clade; WIL = Willughbeieae clade; TAB = Tabernaemontaneae clade; DI = Dyplorhynchus; MEL = Melodineae clade; AMS = Amsonia clade; ALY = Alyxieae clade; HUN = Hunterieae clade; PLU = Plumerieae clade; CAR = Carisseae clade; APSA = Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (retirado de Simões *et al*. 2007).

CAPÍTULO 1

PHYLOGENETIC SYSTEMATICS OF THE TRIBE WILLUGHBEIEAE A. DC. (APOCYNACEAE, RAUVOLFIOIDEAE)

ABSTRACT: Willughbeieae is a pantropical tribe with 18 genera traditionally recognized and 150 species. It comprises species that are mainly trees in the New World and lianas in the Old World and most of species have edible fruits. The aims of the present study are to test the monophyly of Willughbeieae subtribes, to elucidate the phylogenetic relationships among its constituent genera, and to identify morphological synapomorphies to the major clades. We performed phylogenetic analyses using chloroplast (*rpl*16, *rps*16, trnK, *trn*S-G and *mat*K) data from 97 taxa of Willughbeieae, including representatives from all 18 genera. The results of this study support the monophyly of Willughbeieae and of the subtribes: Lacmelleinae, Leuconotidinae, Landolphiinae, Willughbeiinae. We indentified four possible morphological synapomorphies: (1) liana habit; (2) syncarpous ovary; (3) parietal placentation; and (4) baccate fruits. The genera *Ancylobotris, Chamaeclitandra, Clitandra, Cylindropsis, Dyctiophleba, Orthopichonia, Pacouria, Saba* and *Vahadenia* were reduced to *Landolphia*. The necessary new combinations resulting from merging Landolphiinae genera into Landolphia were made. After this work the number of genera in Willughbeieae are nine.

INTRODUCTION

The tropical tribe Willughbeieae is a monophyletic group (Potgieter & Albert, 2001; Sennblad & Bremer, 2002; Simões & al., 2007, 2010), consisting of 18 genera and nearly 150 species, belonging to Rauvolfioideae and form a clade with other two tribes, Tabernaemontaneae and Vinceae (ViWiTa clade, Chapter 2, Simões & al. 2007, 2010). According with Endress & al. (2014), Willughbeieae can be divided into four subtribes: Lacmelleinae Pichon ex Leeuwenb. with four neotropical genera, *Couma* Aubl. (Fig. 1A-D), *Hancornia* Gomes (Fig. 1E), *Lacmellea* H. Karst. (Fig. 2A-D) and *Parahancornia* Ducke (Fig. 2E-F); Landolphiinae K. Schum. with nine paleotropical genera *Ancylobothrys* Pierre (Fig. 3A-B), *Chamaeclitandra* (Stapf) Pichon, *Clitandra* Benth., *Cylindropsis* Pierre, *Dyctyophleba* Pierre, *Landolphia* P. Beauv. (Fig. 3C-D), *Orthopichonia* H. Huber, *Saba* (Pichon) Pichon (Fig. 3E-F), and *Vahadenia* Stapf., and one netropical genus *Pacouria* Aubl. (Fig. 1F); Leuconotidinae Pichon ex Leeuwenb. with two african genera, *Bousigonia* Pierre and *Cyclocotyla* Stapf, and one asian genus *Leuconotis* Jack.; and Willughbeiinae A. DC. with one asian genus *Willughbeia* Roxb.

Willughbeieae was first described by De Candolle (1844), the species were recognized by the following set of characters: calyx without glands, unilocular syncarpic ovary with 2 parietal placenta, amphitropous ovules and seed without coma. De Candolle split it into two subtribes based on fruits and seeds: Allamandeae with capsule, seed marginato-alate and Willughbeiae with berry, naked seeds. According with De Candole, Allamandeae comprises one

genus, *Allamanda* L. and Willughbeiae comprises seven genera: *Carpodinus* R. Br., *Chilocarpus* Blume, *Collophora* Mart., *Couma, Landolphia*, *Pacouria, Willughbeia*. Other genera that belong to Willughbeieae sensu Endress & al. (2014), such as *Hancornia*, *Vahea* Lam. and *Leuconotis,* were placed by De Candolle (1844) in Carisseae, due to bilocular syncarpic ovary.

Currently, *Allamanda* and *Chilocarpus* have been placed in Plumerieae and Alyxieae, respectivelly (Endress & Bruyns 2000); *Collophora* was synonimized in *Couma* by Müeller-Argoviensis (1860), *Vahea* was synonimized in *Landolphia* by Schumann (1893) and *Carpodinus* was synonimized in *Landolphia* by Pichon (1953).

After that, Miers (1878) reduced Willughbeieae to just one genus, *Willughbeia*, based on the absence of albumen. He transfered *Couma*, *Landolphia*, *Pacouria* to Carisseae and *Leuconotis* to Thevetieae. Lacmellea, *Zschokkea* Müll. Arg. and *Clitandra* were added to Carisseae. *Zschokkea* was synonimized in *Lacmellea* by Markgraf (1941).

Since then, the circunscription of the tribe has changed significantly. Schumann (1895) not recognized Willughbeieae, including *Willughbeia* in Arduineae. Arduineae was divided into two subtribes, Melodininae and Landolphiinae, based on bilocular and unilocular ovary, respectively. *Lacmellea* and *Leuconotis* were placed in Melodininae and *Clitandra*, *Couma*, *Hancornia*, *Landolphia* and *Willughbeia* were placed in Landolphiinae.

Pichon (1948) based his tribes classification on fruits characters, the subtribes based on seeds and genera based on flowers features. Carisseae, with nine subtribes, is characterized mainly by fleshy fruits and also corolla tube with a

narrow throat, anthers fully fertile, connective inconspicuous, pollen rarely forming tetrads, style-head without a basal ring, fruits usually indehiscent, seeds without wings. The genera belong to Willughbeieae sensu Endress *et al*. (2014) were placed in five subtribes by Pichon (1948): Couminae comprising *Couma* and *Parahancornia*; Lacmelleinae comprising *Lacmellea* and *Hancornia*; Landolphiinae comprising *Landolphia*, *Pacouria*, *Jasminochyla* M. Pichon (later synonimized in *Landolphia* by Pichon, 1953), *Vahadenia*, *Clitandra* and *Carpodinus* (later synonimized in *Landolphia* by Pichon, 1953); Willughbeiinae comprising *Willughbeia*, *Urnularia* Stapf (later synonimized in Willughbeia by Middleton, 1993); Leuconotidinae comprising *Bousigonia*, *Leuconotis*, *Cyclocotyla*. Couminae differ from others subtribes at hilum shape, which is linear and in the other subtribes is oblong or orbicular. Leuconotidinae have placenta with 1-6 ovules in 1-2 series and cotyledons deeply divided at the base, unlike Lacmelleinae, Landolphiinae and Willughbeiinae that have placenta with 8-∞ ovules in 4-10 series and cotyledons entire or cordate base. Lacmelleinae are trees or shurbs, with pilose style-head, rugose or tuberculate testa, embryo much shorter than the seed, instead of lianes or rhizomatose shrubs, glabrous style-head, embryo as long as the seed in Landolphiineae and Willughbeiinae. The diference between Landolphiinae and Willughbeiinae is in the presence and ausence of endosperm, respectively.

Leeuwenberg (1994) kept the five subtribes proposed by Pichon (1948) and also based his classification on characters of fruits and seeds. He raised some sections to rank of genus, such as *Cylindropsis* in Willughbeiinae,

Chamaeclitandra, *Clitandra*, *Ortopichonia* and *Saba* in Landolphiinae, based on previous Landolphiinae monograph of Pichon (1953).

With molecular phylogenetics studies on Apocynaceae (Endress & al., 1996, Sennblad & Bremer, 1996) Carissaeae was shown to be polyphyletic. The first classification with morphological and molecular evidence proposed by Endress & Bruyns (2000) splits the taxa traditionally ascribed to Carisseae into three tribes (Willughbeieae, Hunterieae and Carisseae s. *str*.).

As currently circumscribed, Willughbeieae comprises species that are mainly trees in the New World and lianas in the Old World. They can be characterized by the presence of white, copious latex in both vegetative and reproductive parts, opposite leaves (whorled in *Couma* Aubl.), inflorescences sometimes with modified grappling or curled tendrils, ovary congenitally syncarpous, mostly unilocular with parietal placentation, style-head usually with stigmatic region beneath a small basal collar, sometimes with the main body uniformly receptive, fruit baccate with a fleshy and nonfibrous pericarp and placentas usually pulpy, seeds usually numerous, embedded in the pulp, and endosperm smooth or wrinkled longitudinally (Endress & Bruyns 2000).

Most of Willughbeieae genera have edible fruits as in neotropical species: *Hancornia speciosa* Gomes, *Couma rigida* Müll. Arg., *Pacouria boliviensis* (Markgr.) A. Chev.; in African species: *Cylindropsis parvifolia* Pierre, *Saba comorensis* (Bojer) Pichon, *Vahadenia laurentii* (De Wild.) Stapf, *Landolphia spp.*; and in Asian species: *Leuconotis eugeniifolia* (Wall. ex G. Don) A. DC., *Willughbeia edulis* Roxb. Other species, such as *Parancornia fasciculata* (Poir.) Benoist in Amazonian forest has edible latex that has been used to treat

respiratory diseases, tuberculosis, asthma, syphilis, anemia, in tradicional comunities and traded in local markets (Shanley & al., 2012).

The aims of the present study are to elucidate the phylogenetic relationships among its constituent genera, to investigate the monophyly of Willughbeieae subtribes, sensu Endress & al. (2014), and to identify morphological synapomorphies to the major clades.

MATERIAL AND METHODS

Taxon sampling, DNA sequencing, alignment

We sampled 97 specimens of Willughbeieae, including representatives from all 18 genera recognized by Endress & al. (2007, 2014) and Simões & al. (2007). The percentage of species sampled were above 60% in twelve genera (*Chamaeclitandra*, *Clitandra*, *Couma*, *Cyclocotyla*, *Cylindropsis*, *Dictyophleba*, *Hancornia*, *Lacmellea*, *Orthopichonia*, *Parahancornia*, *Saba*, *Vahadenia*), 50-59% in three genera (*Ancylobotris*, *Bosigonia*, *Landolphia*) and 30-49% in three genera (*Leuconotis*, *Pacouria*, *Willughbeia*). In addition, twelve taxa from the closely related tribes Vinceae and Tabernaemontaneae were included as outgroups, as well as, two species of Aspidospermeae (*Aspidosperma australe* and *A. cylindrocarpon*) and two species of Alstonieae (*Alstonia scholaris* and *Dyera costulata*). The outgroup sequences were obtained from previous works (Simões & al., 2007, 2010) and also sequences from six species of ingroup (Appendix 1). Taxon names and voucher information are listed in Appendix 2.

DNA extraction, amplification and sequencing were performed following the protocols described in Simões & al. (2007). We used five plastidial regions: *rpl*16, *rps*16 and trnK introns; *trn*S-G intergenic spacer and *mat*K gene. Sequences were aligned using ClustalW version 1.8 (Thompson & al., 1994) and later adjusted visually. Indels were coded as presence/absence states using the simple indel coding method (Simmons & Ochoterena, 2000) as implemented in GapCoder (Young & Healy, 2003) and the coded matrix was appended to the alignment.

Phylogenetic analyses

We searched for incongruence between chloroplast data sets, comparing the topology of all individual data sets based on levels of resolution and bootstrap support. In the absence of incongruence all plastid regions were combined.

Phylogenetic trees of the combined matrix of the five plastid DNA markers and indels coded were reconstructed using maximum parsimony (MP, Fitch, 1971) and Bayesian inference (BI, Mau & al. 1999). The MP analyses were conducted in PAUP* 4.0b (Swofford, 2002). Heuristic search included an initial round of tree searches with 1000 randon addition sequence replicates, holding 10 trees at each step, tree bisection-reconnection (TBR) and branch swapping with MULTREES. All trees retained in the memory were then included in a second round of searches. All characters were unordered and equally weighted. Bootstrap analyses were run based on 2000 pseudoreplicates, each with one random taxon addition replicate followed by TBR branch swapping. We consider

75–84% bootstrap values moderate support and 85–100% strong support (Muellner & al. 2008).

Bayesian analyses were carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Two independent runs of Markov Chain Monte Carlo (MCMC) with four chains (one heated and three cold), were run for twenty million generations, sampling trees every 2000 generations. After discarding 25% of the saved trees as burn-in, a maximum credibility tree based on remaining trees was produced using Tree Anotator v1.7.4 (Drummond & al., 2012) and exported to Fig Tree 1.4.0 (Rambaut, 2012). Posterior probabilities ≥ 0.95 were considered strongly supported (Alfaro & al., 2003; Huelsenbeck & Rannala, 2004).

An appropriate substitution model was selected to each partition based on the Bayesian Information Criterion (BIC) using jModeltest v. 3.6 (Posada, 2008). The substitution model choosen to indels in MrBayes analyses was a simple model, where all substitutions have the same rate, corresponding to a Jukes-Cantor model.

Morphological ancestral character states reconstruction

Binary and multistate characters were scored in order to identify synapomorphies that are congruents with each of major clades of Willughbeieae. Characters were coded based on examination of herbarium specimens (BR, IAN, INB, INPA, L, MG, UEC, WAG, Z) and literature (e.g., Monachino, 1943, 1944, 1945a, 1945b; Pichon, 1953; Van der Ploeg, 1985; Leeuwenberg & Berndsen, 1988; Hoogh, 1989; Leeuwenberg & van Dilst, 1989; Vonk, 1989; Persoon, & al., 1992; Vonk, & al. 1994; Middleton, 1993, 2007, 2011;

Haegens, 1994; van Dilst, 1999; Leeuwenberg, 2002). Character evolution was reconstructed using 100 trees generated in Bayesian analysis, chosen randomly. Ancestral state reconstructon was summarized in a maximum clade credibility tree. The parsimony criteria as implemented in Mesquite 2.75 (Maddison & Maddison, 2011) was selected and all characters were considered unordered and unweighted.

RESULTS

Phylogenetic analysis

The aligned total evidence matrix contains 6411 characters. The aligned lenghts of *rpl*16, *rps*16 and *trn*K introns; *trn*S-G intergenic spacer; *mat*K gene and indels datasets were 1245, 961, 1136, 938, 1597, 534 respectively. The MP analyses of combined matrix resulted in 3867 constant sites, 1248 variable sites but parsimony-uninformative, and 1296 parsimony-informative sites. The tree lenght was 3558 steps, consistency index (CI) was 0.460, and retention index (RI) was 0.736. The results of Parsimony analysis are depicted onto the strict consensus tree with bootstrap support (BS) values higher than 50% (Fig. 4).

The best-fit model of sequence evolution was TPM3uf+G to *trn*S-G, TPM1uf+G to *rpl*16 and *rps*16, TVM+G to *mat*K and *trn*K. The results of the Bayesian analysis based on the combined data including the indel matrix are presented on the maximum clade credibility tree with posterior probabilities (PP) values (Fig. 5, 6).

The monophyly of Willughbeieae sensu Simões & al. (2007) is highly supported (BS=89%, PP=1, Fig.4, Fig. 5). Relationships between Willughbeieae and other tribes of Rauvolfioideae are still uncertain. The sampled representatives of Tabernaemontaneae were found to be sister to Willughbeieae in the bayesian analysis (PP=0.84), whereas a sister relationship with the representatives of Vinceae was recovered in the parsimony analysis (BS=60%), both with low support. Within Willughbeieae four major clades that largely correspond to the subtribes proposed by Endress & al. (2014) were found. Clade LEUCO (BS=90%, PP=1) comprises *Bousigonia*, *Cyclocotyla* and *Leuconotis* genera. Clade LACME (BS=83%, PP=0.78) comprises all sampled specimens of the four Neotropical genera (*Couma*, *Hancornia*, *Lacmellea* and *Parahancornia*). Clade WILLU (BS=99, PP=1) encompasses species from the paleotropical genus *Willughbeia*. Clade LANDO (BS=71%, PP=0.99) is the largest one, comprising representatives of nine genera. *Clitandra*, monotipic, is the first lineage to diverge. The largest genus of the tribe, *Landolphia* is polyphyletic as well *Dictyophleba* and *Ancylobotris*. *Pacouria*, the only Neotropical genera in this clade, is nested within *Landolphia*.

Morphological ancestral character states reconstruction

The characters and character states are given in Appendix 3. The complete morphological matrix with 40 characters codified for the 97 ingroup taxa plus outgroup is presented in Appendix 4. From the 40 optimized morphological characters, 18 were reconstructed as potential synapomorphies for major clades of Willughbeieae (Figs. 8 to 21).

DISCUSSION

Willughbeieae and its four subtribes, as circumscribed by Endress & al. (2014), are resolved as monophyletic. In this study we cannot confirm whether Tabernaemontaneae or Vinceae is the sister group of Willughbeieae, as well in the others phylogenetics reconstructions of Tabernaemontaneae and Vinceae (Simões & al., 2010; data not publis.). The uncertainty in Willughbeieae sister group could be result of long-branch attraction, as observed some long branches (Fig. 6) in Vinca_minor, stem branch of Tabernaemontaneae (TABER) and stem branch of Leuconotidinae (LEUCO). Among the methods suggested by Bergsten (2005) to avoid long-branch attraction is sampling more taxa to break-up long branches, suggestion that was followed in the Chapter 2, where we analysed a combined matrix with 288 taxa of ViWiTa clade, and found Tabernaemontaneae, as sister-group to Willughbeieae with high support, PP=0.98 (Fig. 3 in Chapter 2). The comparison between Willughbeieae and ViWiTa phylograms is presented in Fig. 7, and possible long-branch attraction in Willughbeieae phylogram in Fig. 7A.

Willughbeieae is supported by six potential morphological synapomorphies: (1) liana habit (Fig. 8); (2) style-head body apex undifferentiated (Fig. 11); (3) style-head body base undifferentiated (Fig. 12); (4) syncarpous ovary (Fig. 13); (5) parietal placentation (Fig. 18); and (6) baccate fruits (Fig 20). The reconstruction of habit charater is ambiguous: the MRCA of Willughbeieae could have been a liana and changed to tree or shrub and then

changed to liana in parallel in the LANDO+WILLU clade and LEUCO clade, or the MRCA could be a liana and change to tree or shrub in LACME clade.

Subtribe Leuconotidinae

Leuconotidinae is monophyletic (PP=1) and is the first lineage to diverge within Willughbeieae. It comprises all genera (*Bousigonia*, *Cyclocotyla*, *Leuconotis*) of Leuconotidinae sensu Pichon (1948), Leeuwenberg (1994), Endress & al. (2014). We found three possible synapomorphies to this subtribe, endosperm lacking or scanty (caracter 37, Fig. 21), 1-10 ovules per carpel (character 28, Fig. 17), and inner surface of the calyx with standard colleters (caracter 8, Fig. 10).

Representatives Leuconotidinae are lianas without tendrils. *Bousigonia,* with two species and *Leuconotis,* with four species occur in tropical Asia while *Cyclocotyla*, monotypic, is restricted to tropical Africa (Leeuwenberg, 2002). The monophyly of *Bousigonia* cannot be tested because just one species was sampled. Two species of *Leuconotis* were sampled and *L. anceps* positioned as the sister group to *L. eugeniifolia* and *Cyclocotyla congolensis* in Bayesian and parsimony analyses, wich suggest that *Leuconotis* may be paraphyletic. There is no information in the literature on this relationship. However, *Leuconotis* and *Cyclocotyla*, they share a morphological character the bilocular ovary, while in *Bousigonia* it is unilocular (Van der Ploeg, 1985; Shu, 1995; Leeuwenberg, 2002).

The flowers of *Leuconotis* are tetramerous and a nectary disc is lacking, while the other two genera have pentamerous flowers and a conspicuous nectary disk (Leeuwenberg, 2002).

Subtribe Lacmelleinae

The subtribe comprises four neotropical monophyletic genera: *Couma* (PP=1, BS=93%), *Hancornia* (PP=1, BS=100%), *Lacmellea* (PP=1, BS=74%) and *Parahancornia* (PP=1, BS=100%). These genera can be easily recognized by morphological caracters. *Couma* is the only genus of Willughbeieae with whorled leaves and lilac to pinkish flowers, while all other genera have opposite leaves and whitish flowers. *Lacmellea,* on the other hand, is the only Neotropical genus of Willughbeieae with axillary inflorescences with a spinescent appendage at the fruit apex, formed by the lignified basal portion of the style. *Parahancornia* is recognized by 4-merous calyx and terminal inflorescences, usually with many flowers.

Lacmellea and *Couma* are sisters groups (PP= 1, BS=89%), unlike of hypothesizes by Pichon (1948) and Leeuwenberg (1994) that accounted *Couma* and *Parahancornia* under Couminae due to linear hilum, hemisyncarpous ovary (fused at the base), pericarp without a sclerified layer, seeds with a deep ventral groove, embryo slightly shorter than the seed, and *Hancornia* and *Lacmellea* under Lacmelleinae due to habit (trees or shrubs), pilose style-head, rugose or tuberculate testa, embryo much shorter than the seed, and pericarp without a sclerified layer.

Hancornia was supported as the sister group to *Parahancornia* in Bayesian and parsimony analysis, but with weak and moderate support (PP=0.65, BS=83%). Ducke (1922) described the genus *Parahancornia* on the basis of *Hancornia amapa* Huber and suggested that it should be placed next to *Hancornia* and

Couma. Monachino (1945) stated that *Hancornia* should be placed next to *Parahancornia*.

A possible morphological synapomorphy to the Lacmelleinae is the habit (tree or shrubs) if we consider liana habit as the state of MRCA of Willughbeieae.

Subtribe Willughbeiinae

Willughbeiinae is monophyletic (PP=1) and comprises the genus *Willughbeia* (Pichon, 1948; Endress & al., 2014). Pichon (1953) excluded *Cylindropsis* from Landolphiinae due to lack of endosperm, while Leeuwenberg (1994) maintained this arrangement. Haegens (1994) observed in *Cylindropsis* and *Willughbeieae* a very thin endosperm. In our results *Cylindropsis* is nested within LANDO clade, and therefore do not support the circunscription of Willughbeiinae by Pichon (1953) and Leeuwenberg (1994). We found two possible synapomorphies to the group: the half-inferior position of the ovary (caracter 24, fig. 14) and endosperm lacking or scanty. The half-inferior position of the ovary originated in parallel in *Couma* and *Orthopichonia*, and the endosperma lacking or scanty originated in parallel in *Cylindropsis* and in Leuconotidiinae (character 37, Fig. 21).

Willughbeia has 16 species distributed in Southeastern Asia, comprising lianas with tendrils formed from modified inflorescences, fleshy berry, few to many seeded; indehiscent, seed ovoid, without couma; smooth with a very thin endosperm and thick horny cotyledons (Middleton, 1993).

Subtribe Landolphiinae

Landolphiinae sensu Leeuwenberg (1994) and Endress & al., (2014) is monophyletic. However the biggest genus of subtribe, *Landolphia*, sensu Pichon (1953) and Persoon & al. (1992) is paraphyletic. Pichon (1953), in his important monograph about Landolphiinae, discussed the problem of finding morphological features to caracterize the genera. He considered three solutions to solve this problem: 1. put all genera under *Landolphia*, 2. split *Landolphiinae* in minor genera until the point to have exclusive and stable characters, 3. change the generic limits. He choosed the second and third options and accepted eleven genera, although emphasizing that the morphological differences between genera were minimum. Persoon & al. (1992) stated that the Landolphiinae genera are all closely allied to each other by almost all characters. They recognized nine genera from eleven of Pichon (1953), *Anthoclitandra* and *Aphanostylis* were synonymized within *Landolphia*.

Diagnostic characters used by Pichon (1953) to delimit Landolphiinae genera were: number of functional pores in pollen grains (3-4 pores vs. 1); style-head (shape and size); inflorescence type and position (cymes grouped or not in panicles, terminal or axillary); stipules (present vs. absent); calycine colleters (present vs. absent); corolla structure (thickened or not between stamens and throat, length of the tube, lobes cilliate or not); anthers (carennate or not, length and proportion to the corolla tube, insertion level); ovary (glabrous vs. pilose, number of series of ovules); fruit (glabrous vs. pubescent, presence or not of a sclerified layer at the pericarp).

Persoon & al., (1992) used the same caracteres used by Pichon (1953) to define the Landolphiinae genera.

Considering the resuts from our phylogenetic analyses, we propose a new circunscription to *Landolphia* with the inclusion of *Ancylobotris, Chamaeclitandra, Clitandra, Cylindropsis, Dyctiophleba, Orthopichonia, Pacouria, Saba* and *Vahadenia* into *Landolphia* sensu lato (s.l.).

Landolphia s.l. has an African continental distribution with fifty species, six species endemic to Madagascar and three species in the Neotropics. These disjunction could indicate a colonization event from Paleotropics to the Neotropics.

Taxonomic consequences

Willughbeieae A. DC. *in* DC. & A. DC., Prodr. 8: 318. 1844.

Type: Willughbeia Roxb., nom. cons. 1820. 9 genera.

Trees, shrubs, or lianas, the last often with grappling tendrils, with milky latex. Leaves opposite (whorled in *Couma*); calycine colleters present or absent; corolla salverform; corolline corona absent; corolla lobe aestivation almost always sinistrorse; stamens included; apical connective appendages of anthers often scarcely developed; style-head usually without basal collar, sometimes body uniformely receptive; ovary congenitally syncarpous; disc mostly absent, when present adnate or indistinct from ovary. Fruit indehiscent, a berry with fleshy, non-fibrous pericarp and placenta becoming pulpy in fruit; seeds ovoid, embedded in pulp, with or without a longitudinal furrow; endosperm smooth or wrinkled longitudinally. Pollen (1 to)3(to 4)-colporate. Secondary chemistry indole alkaloids. (Description extracted from Simões & al., 2007)

Subtr. Lacmelleinae Pichon ex Leeuwenb., Wageningen Agric. Univ. Pap. 94(3): 54.1994. (Lacmelliinae). — Type: *Lacmellea* Karst. 1857.

Couma Aubl., Hist. Pl. Guiane Suppl.: 39, t. 392. 1775.

Hancornia Gomes, Observ. Bot.-Med. Nonnullis Bras. Pl. 2: 1, t. 1. 1803.

Lacmellea H. Karst., Linnaea 28: 449. 1857.

Parahancornia Ducke, Arch. Jard. Bot. Rio de Janeiro 3: 242. 1922.

Subtr. Landolphiinae K. Schum. *in* Engl. & Prantl, Nat. Pflanzenfam. 4(2): 122, 127. 1895 — Type: *Landolphia* P. Beauv. 1806.

Landolphia P. Beauv., Fl. Oware Bénin 1: 54. 1804, nom. cons.

Syn: *Ancylobotrys* Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 91. 1898.; *Chamaeclitandra* (Stapf) Pichon, Mém. Inst. Franç. Afrique Noire 35: 202. 1953.; *Clitandra* Benth *in* Hook., Nig. Fl. 445. 1849.; *Cylindropsis* Pierre, Bull. Mens. Soc. Linn. Paris, ser. 2, 1: 38. 1898.; *Dictyophleba* Pierre, Bull. Mens. Soc. Lin. Paris, ser. 2, 1: 92. 1898.; *Ortopichonia* H. Huber, Kew Bull. 15(3): 437. 1962.; *Pacouria* Aubl., Hist. Pl. Guiane 268. 1775; *Saba* (Pichon) Pichon, Mém. Inst. Franç. Afrique Noire 35: 302. 1953.; *Vahadenia* Stapf *in* Dyer, Fl. Trop. Afr. 4(1): 29. 1902.

Subtr. Leuconotidinae Pichon ex Leeuwenb., Wageningen Agric. Univ. Pap. 94(3): 53. 1994. — Type: *Leuconotis* Jack 1823.

Bousigonia Pierre, Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 35. 1898.

Cyclocotyla Stapf, Bull. Misc. Inform. 1908: 259. 1908.

Leuconotis Jack, Trans. Linn. Soc. 14: 121. 1823.

Subtr. Willughbeiinae A. DC. *in* DC. & A. DC., Prodr. 8: 319. 1844. — Type: *Willughbeia* Roxb. 1820, nom. cons.

Willughbeia Roxb., Pl. Coromandel 3: 77, t. 280. 1820, *nom. cons.*

New combinations

Landolphia sp. R. Morokawa & A.O. Simões, **nom. nov. ≡** *Cylindropsis parvifolia* Pierre, Bull. Mens. Soc. Linn. Paris, ser. 2, 1: 38. 1898. **≡** *Clitandra parvifolia* (Pierre) Stapf in Dyer, Fl. Trop. Afr. 4(1): 63. 1902 – Type: Gabon, near Libreville, 3 May 1897, *Klaine 103bis* (lectotype, BR; isotypes: K, P)

A new species name is necessary, since the name *Landolphia parvifolia* K. Schum. (1859) already exists.

Landolphia barteri (Stapf) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia barteri* (Stapf) H. Huber, Kew Bull. 15: 437. 1962. **≡** *Clitandra barteri* Stapf, Bull. Misc. Inform. Kew 1894(1): 20. 1894.

Landolphia cirrhosa (Radlk.) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia cirrhosa* (Radlk.) H. Huber, Kew Bull. 15: 437. 1962. **≡** *Clitandra cirrhosa* Radlk., Abh. Naturwiss. Vereine Bremen 8: 400. 1883.

Landolphia indeniensis (A. Chev.) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia indeniensis* (A. Chev.) H. Huber, Kew Bull. 15: 437. 1962. **≡** *Clitandra indeniensis* A. Chev., Mém. Soc. Bot. France 8: 43. 1908.

Landolphia schweinfurthii (Stapf) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia schweinfurthii* (Stapf) H. Huber, Kew Bull. 15: 437. 1962. **≡** *Clitandra schweinfurthii* Stapf, Bull. Misc. Inform. Kew 1894(1): 20. 1894.

Landolphia seretii (De Wild.) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia seretii* (De Wild.) Vonk, Agric. Univ. Wageningen Pap. 89(4): 43. 1989. ≡ *Clitandra seretii* De Wild., Not. Pl. Util. Congo 2: 226. 1908.

Landolphia setosa (de Hoogh) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Dictyophleba setosa* de Hoogh, Bull. Jard. Bot. Belg. 59: 220. 1989.

Landolphia visciflua (K. Schum. ex Hallier f.) R. Morokawa & A.O. Simões, **comb. nov. ≡** *Orthopichonia visciflua* (K. Schum. ex Hallier f.) Vonk, Agric. Univ. Wageningen Pap. 89(4): 46. 1989. ≡ *Clitandra visciflua* K. Schum. ex Hallier f., Jahrb. Hamburg. Wiss. Anst. 17: 127. 1900.

Restored names

Landolphia boliviensis Markgr., Notzibl. Bot. Gat., Berlin-Dahlem 9: 1041. 1926. ≡ *Pacouria boliviensis* (Markgr.) A. Chev., Rev. Int. Bot. Apll. Agric. Trop. 28: 455. 1948.

Landolphia caillei A. Chev., *in* Vilbouchevitch, Journ. Agr. Trop. 6: 159. 1906. ≡ *Vahadenia caillei* A. Chev., (A. Chev.) Stapf ex Hutch & Dalz. *in* Dyer, Fl. W. Trop. Afr. 2: 31. 1931.

Landolphia capensis Oliv., Hooker's Icon. Pl. 13: 22., t. 1228. 1877. ≡ *Ancylobotrys capensis* (Oliv.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 284. 1953.

Landolphia comorensis (Bojer ex A.DC.) K. Schum., Bot. Jahrb. Syst. 15: 402. 1893. ≡ *Saba comorensis* (Bojer ex A.DC.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 303. 1953.

Landolphia henriqueziana Hallier f., Jahrb. Hamburg. Wiss. Anst. 17: 97. 1899. ≡ *Chamaeclitandra henriqueziana* (Hallier f.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 203. 1953.

Landolphia laurentii De Wild, Rev. Cult. Col. 8: 229. 1901. ≡ *Vahadenia laurentii* (De Wild) Stapf in Dyer, Fl. Trop. Afr. 4(1): 30. 1902.

Landolphia leonensis Stapf, in Dyer, Fl. Trop. Afr. 4(1): 36. 1902. ≡ *Dictyophleba leonensis* (Stapf) Pichon, Mém. Inst. Franç. Afrique Noire 35: 256., t. 14, 1-4. 1953.

Landolphia lucida K. Schum., Notzibl. Bot. Gart. Berlin 1: 24. 1895. ≡ *Dictyophleba lucida* (K. Schum.) Pierre, Bull. Mens. Soc. Linn. Paris, ser. 2, 1: 93. 1898.

Landolphia ochracea K. Schum. ex Hallier f., Jahrb. Hamburg. Wiss. Anst. 17(3), Beih. 86. 1900. ≡ *Dictyophleba ochracea* (K. Schum. ex Hallier f.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 251. 1953.

Landolphia paraensis Huber, Bol. Mus. Goeldi Hist. Nat. Ethnogr. 6: 88-89. 1910 ≡ *Pacouria paraensis* (Huber) Pichon, Mém. Mus. Natl. Hist. Nat. 24: 144. 1948.

Landolphia petersiana (Klotzsch) Dyer, Kewe Gard. Rep. 1880: 42-43. 1881. ≡ *Ancylobotrys petersiana* (Klotzsch) Pierre, Bull. Mens. Soc. Linn. Paris 1: 91. 1898.

Landolphia reticulata Hallier f., Jahrb. Hamburg. Wiss. Anst. 17: 87. 1900. ≡ *Ancylobothrys reticulata* (Hallier f.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 300. 1953.

Landolphia robusta (Pierre) Stapf, Fl. Trop. Afr. 4(1): 43. 1902. ≡ *Ancylobotrys robusta* Pierre, Bull. Mens. Soc. Linn. Paris, ser 2: 128. 1899.

Landolphia scandens (Schumach. & Thonn.) Didr., Vidensk. Meddel. Danks Naturhist. Foren. Kjobenhavn 1853: 190. 1854. ≡ *Ancylobothrys scandens* (Schumach. & Thonn.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 286. 1953.

Landolphia senegalensis (A.DC.) Kotschy & Peyr., Pl. Tinn. 31. 1867. ≡ *Saba senegalensis* (A.DC.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 316. 1953.

Landolphia stipulosa S. Moore ex Wernham, Cat. Pl. Oban 59. 1913. ≡ *Dictyophleba stipulosa* (S. Moore ex Wernham) Pichon, Mém. Inst. Franç. Afrique Noire 35: 259. 1953.

Landolphia tayloris Stapf, Fl. Trop. Afr. 4(1): 45. 1902. ≡ *Ancylobothrys tayloris* (Stapf) Pichon, Mém. Inst. Franç. Afrique Noire 35: 284. 1953.

Landolphia thompsonii A. Chev., Mém. Soc. Bot. France 8: 182. 1912. ≡ *Saba thompsonii* (A. Chev.) Pichon, Mém. Inst. Franç. Afrique Noire 35: 322, t. 21, 4-6. 1953.

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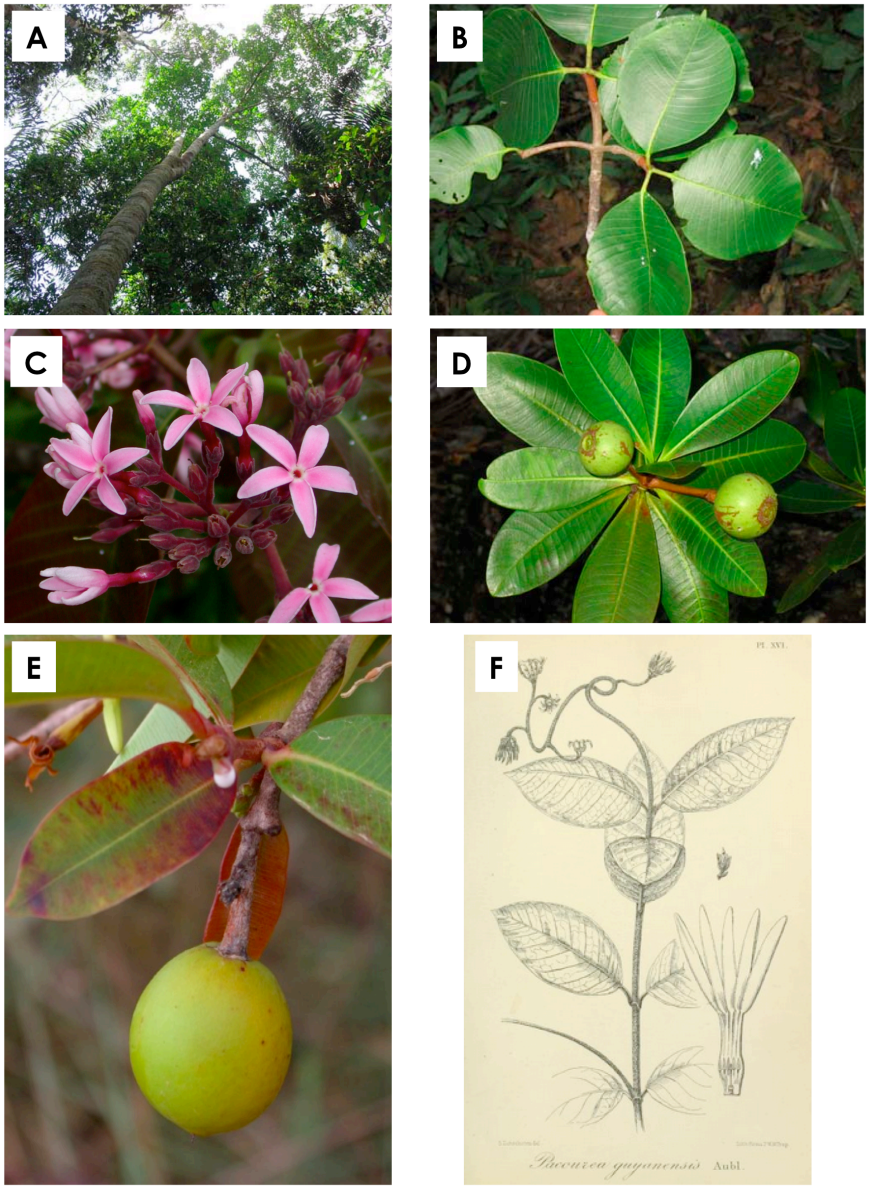


Fig. 1. Examples of Willghbeieae species. A-C: *Couma macrocarpa* A: an individual in Amazonian forest; B: branch with verticillate leaves; C: inflorescence; D: *Couma rigida*, fruits; E: *Hancornia sperciosa*, fruit; F: *Pacouria guianensis*, branch with inflorescence tendril-like and flower details.

(A,B: Manaus, AM; C: Boca San Carlos, Costa Rica; D: Mucugê, BA. E: Itabaiana, SE. Fotos: A,B, D: A.O. Simões; C, E: J.F. Morales. Illustration: F: Pulle,1906)

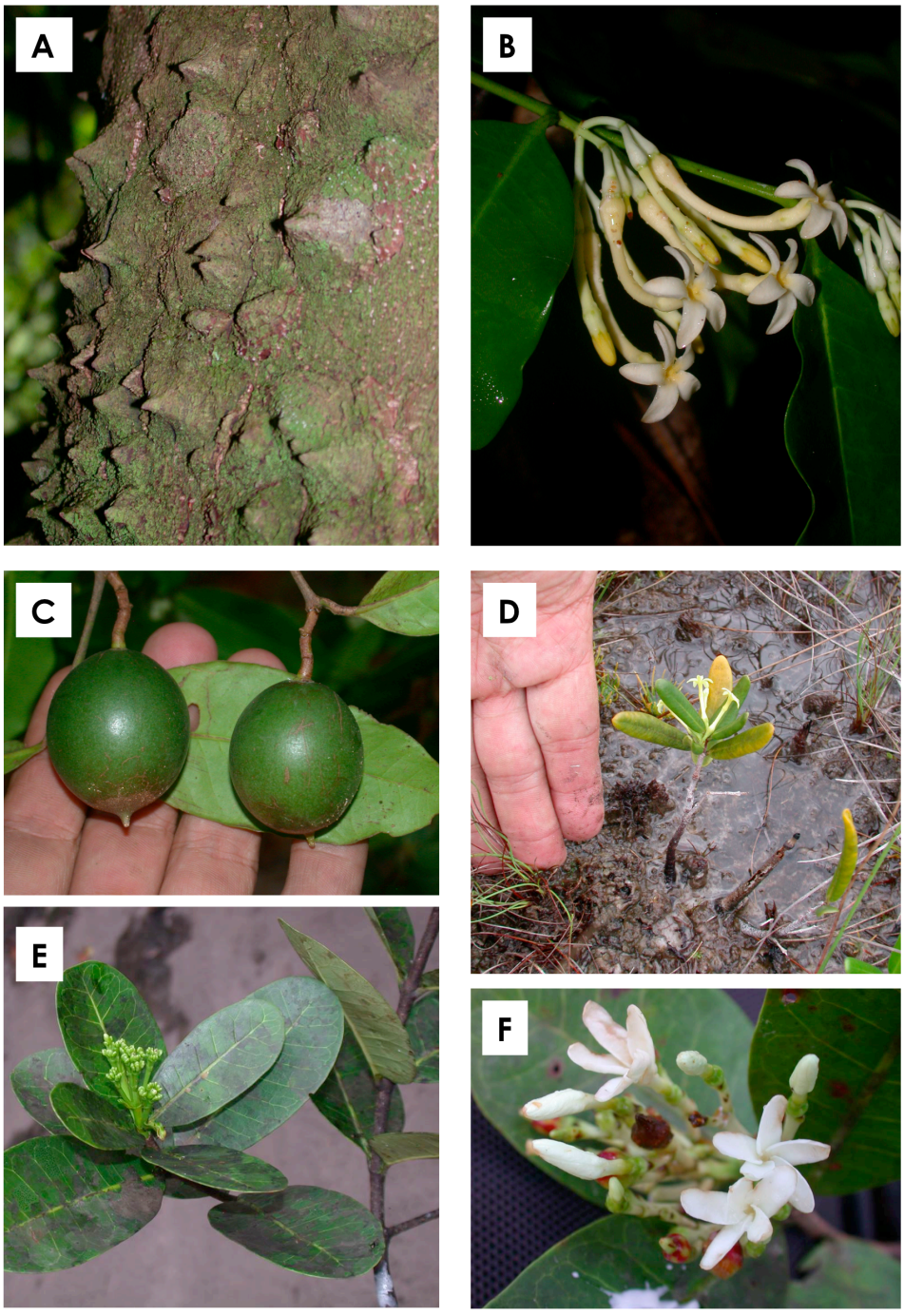
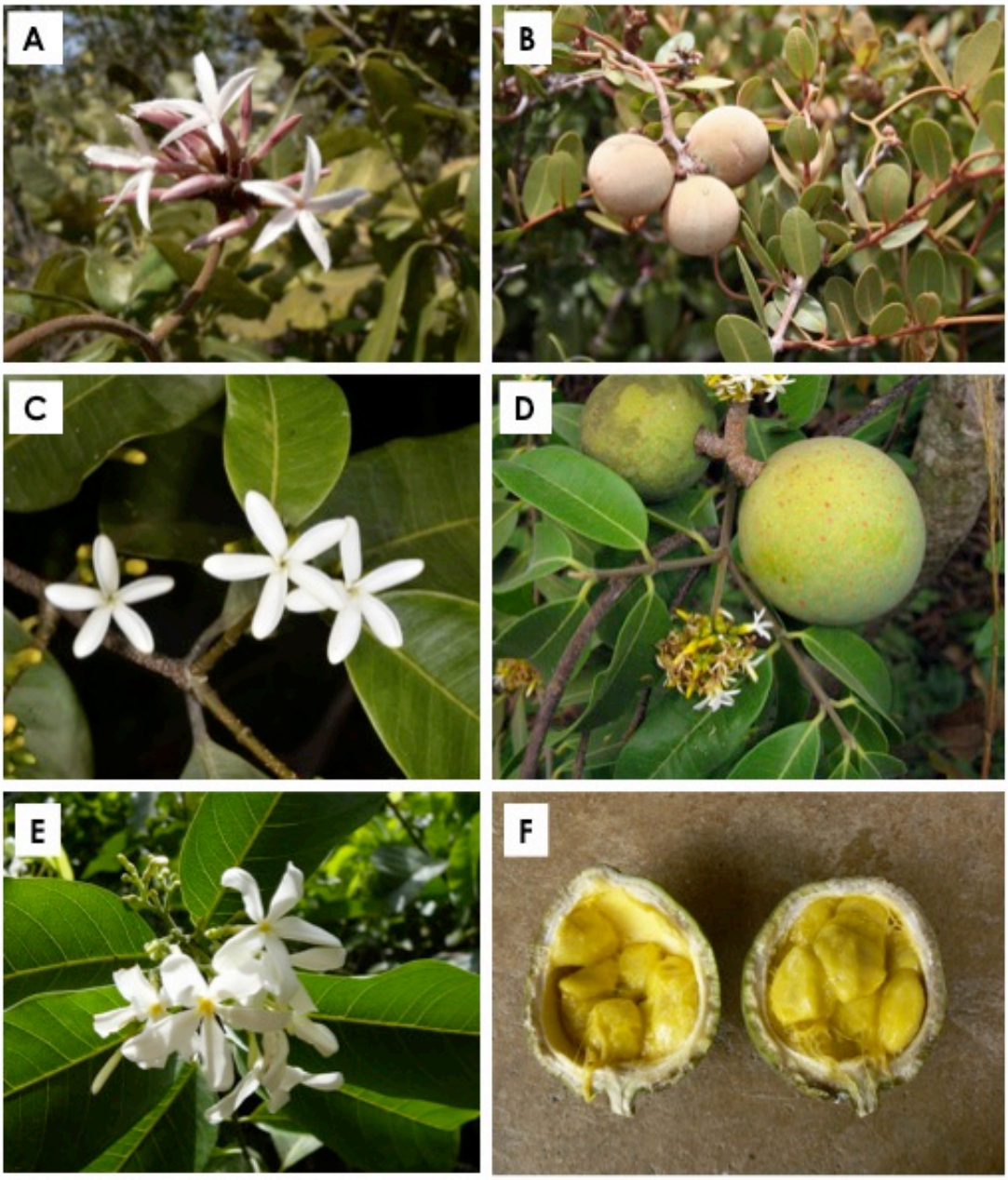


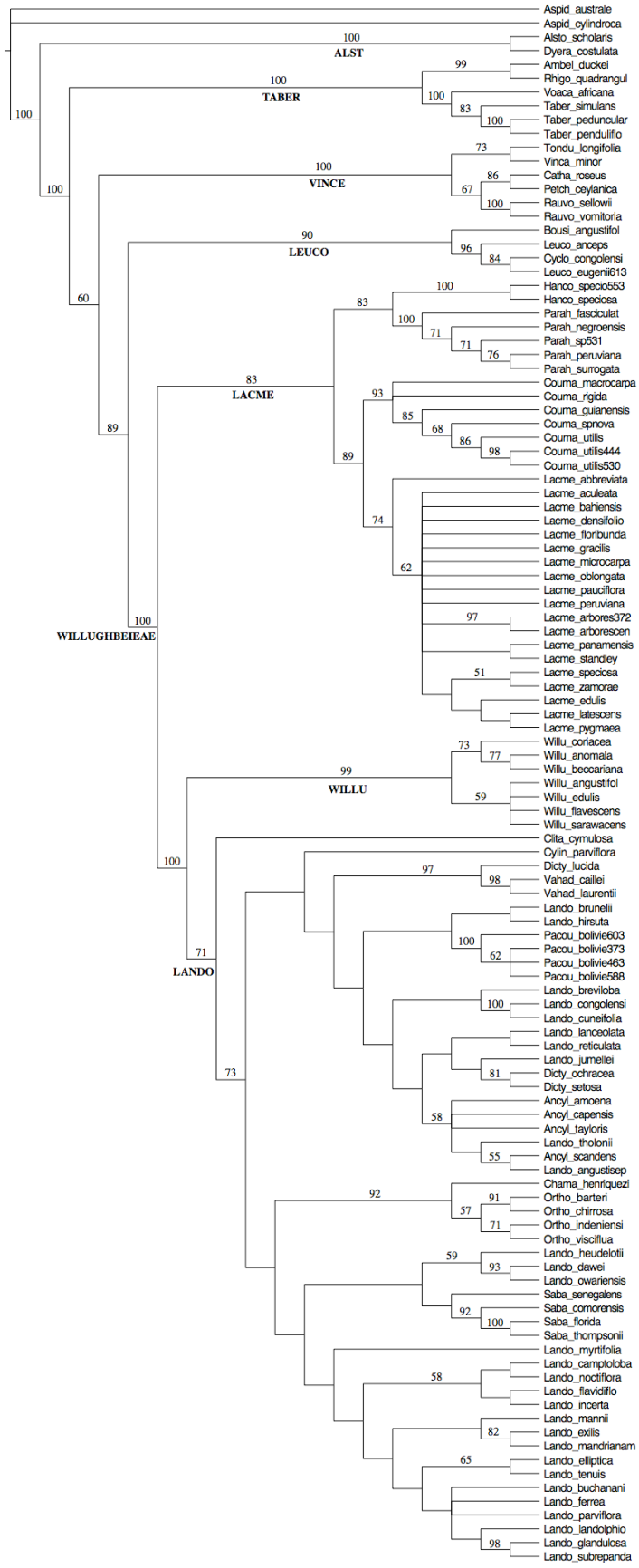
Fig. 2. Examples of Willughbeieae species. A-C: *Lacmellea panamensis.* A: detail of trunk with evidence of prickles; B: inflorescence; C: fruits. D: *Lacmellea pygmaea*, habit. E-F: *Parahancornia fasciculata*. E: branch with opposite leaves and young inflorescence; F: inflorescence.

(A-C: Osa, Costa Rica; D: Venezuela, E,F: Guainia, Colombia. Fotos: J.F. Morales)



Illustrations of Willughbeieae species. A: *Ancylobotris amoena,* inflorescence branch; B: *Ancylobotris capensis,* fruits; C: *Landolphia buchananii,* flowers; D: *Landolphia kirkii*, inflorescence branch and fruits; E: *Saba senegalensis*, inflorescence branch; F: *Saba comorensis*, open fruit.

(A, F: Günter Baumann; B: Marco Schmidt; C: Matthew Walters; D: Robert v. Blittersdorff; E: Andreas Tervort).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Dyera_costulata
Ambel_duckei
Rhigo_quadrangul
Voaca_africana
Taber_simulans
Taber_peduncular
Taber_penduliflo
Tondu_longifolia
Vinca_minor
Catha_roseus
Petch_ceylanica
Rauvo_sellowii
Rauvo_vomitoria
Bousi_angustifol
Leuco_anceps
Cyclo_congolensi
Leuco_eugenii613
Hanco_specio553
Hanco_speciosa
Parah_fasciculat
Parah_negroensis
Parah_sp531
Parah_peruviana
Parah_surrogata
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Couma_rigida
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Couma_utilis530
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Lacme_aculeata
Lacme_bahiensis
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Lacme_floribunda
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Lacme_oblongata
Lacme_pauciflora
Lacme_peruviana
Lacme_arbores372
Lacme_arborescen
Lacme_panamensis
Lacme_standley
Lacme_speciosa
Lacme_zamorae
Lacme_edulis
Lacme_latescens
Lacme_pygmaea
Willu_coriacea
Willu_anomala
Willu_beccariana
Willu_angustifol
Willu_edulis
Willu_flavescens
Willu_sarawacens
Clita_cymulosa
Cylin_parviflora
Dicty_lucida
Vahad_caillei
Vahad_laurentii
Lando_brunelii
Lando_hirsuta
Pacou_bolivie603
Pacou_bolivie373
Pacou_bolivie463
Pacou_bolivie588
Lando_breviloba
Lando_congolensi
Lando_cuneifolia
Lando_lanceolata
Lando_reticulata
Lando_jumellei
Dicty_ochracea
Dicty_setosa
Ancyl_amoena
Ancyl_capensis
Ancyl_tayloris
Lando_tholonii
Ancyl_scandens
Lando_angustisep
Chama_henriquezi
Ortho_barteri
Ortho_chirrosa
Ortho_indeniensi
Ortho_visciflua
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Lando_dawei
Lando_owariensis
Saba_senegalens
Saba_comorensis
Saba_florida
Saba_thompsonii
Lando_myrtifolia
Lando_camptoloba
Lando_noctiflora
Lando_flavidiflo
Lando_incerta
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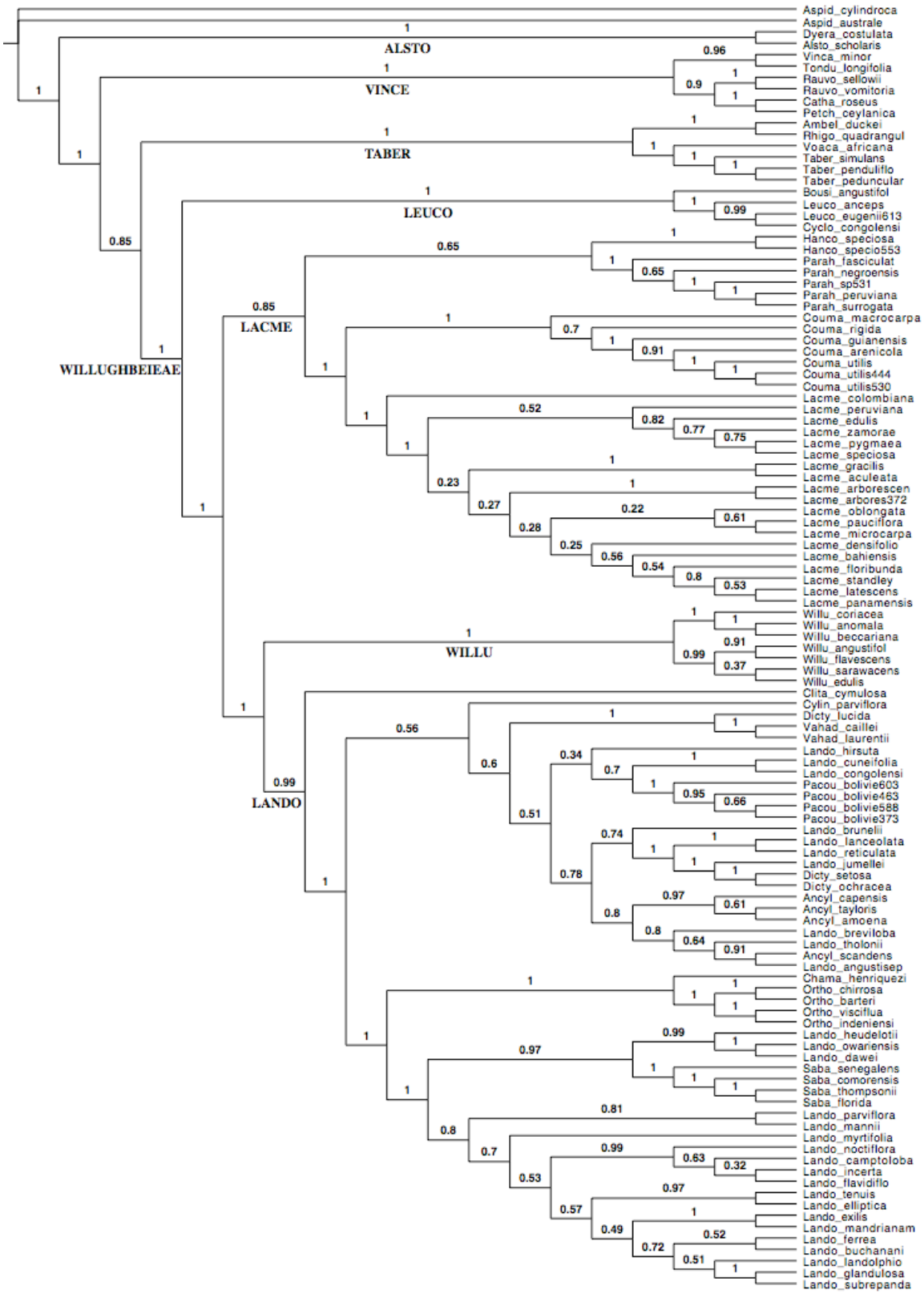
Lando_exilis
Lando_mandrianam
Lando_elliptica
Lando_tenuis
Lando_buchanani
Lando_ferrea
Lando_parviflora
Lando_landolphio
Lando_glandulosa
Lando_subrepanda
ALST
TABER
VINCE
LEUCO
LACME
WILLUGHBEIEAE
WILLU
LANDO

Fig. 4. Strict consensus tree based on the Parsimony analysis of combined molecular dataset (*rpl*16, *rps*16, trnK, *trn*S-G and *mat*K). Bootstrap support are indicated above branches. Full taxon names are given in the Appendix 1. ALSTO = Alstonia; TABER = Tabernaemontaneae; VINCE = Vinceae; LEUCO= Leuconotinae; LACME = Lacmeliinae; WILLU = Willughbeiinae; LANDO = Landolphiinae.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Rauvo_vomitoria
Catha_roseus
Petch_ceylanica
Ambel_duckei
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Leuco_eugenii613
Cyclo_congolensi
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Ancyl_capensis
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Lando_exilis
Lando_mandrianam
Lando_ferrea
Lando_buchanani
Lando_landolphio
Lando_glandulosa
Lando_subrepanda
ALSTO
VINCE
TABER
LEUCO
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WILLUGHBEIEAE
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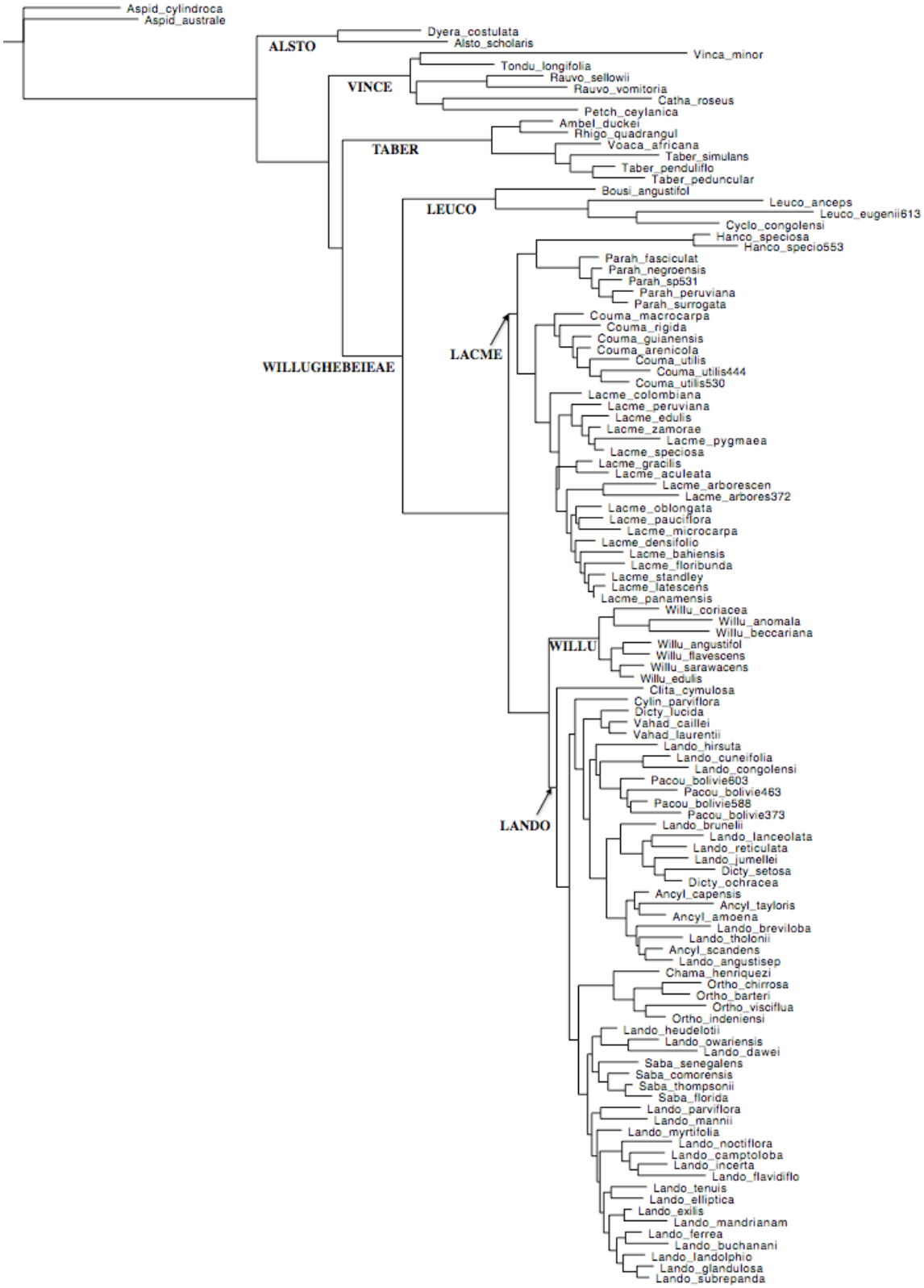
LANDO

Fig. 5. Maximum clade credibility tree based on the Bayesian analysis of combined molecular dataset (*rpl*16, *rps*16, trnK, *trn*S-G and *mat*K). Posterior probabilities are indicated above branches. Full taxon names are given in the Appendix 1. ALSTO = Alstonia; TABER = Tabernaemontaneae; VINCE = Vinceae; LEUCO= Leuconotinae; LACME = Lacmeliinae; WILLU = Willughbeiinae; LANDO = Landolphiinae.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Rauvo_vomitoria
Catha_roseus
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Rhigo_quadrangul
Voaca_africana
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Lando_elliptica
Lando_exilis
Lando_mandrianam
Lando_ferrea
Lando_buchanani
Lando_landolphio
Lando_glandulosa
Lando_subrepanda

Fig. 6. Phylogram derived from a maximum clade credibility tree based on the Bayesian analysis of combined molecular dataset (*rpl*16, *rps*16, trnK, *trn*S-G and *mat*K). Full taxon names are given in the Appendix 1. ALSTO = Alstonia; TABER = Tabernaemontaneae; VINCE = Vinceae; LEUCO= Leuconotinae; LACME = Lacmeliinae; WILLU = Willughbeiinae; LANDO = Landolphiinae.

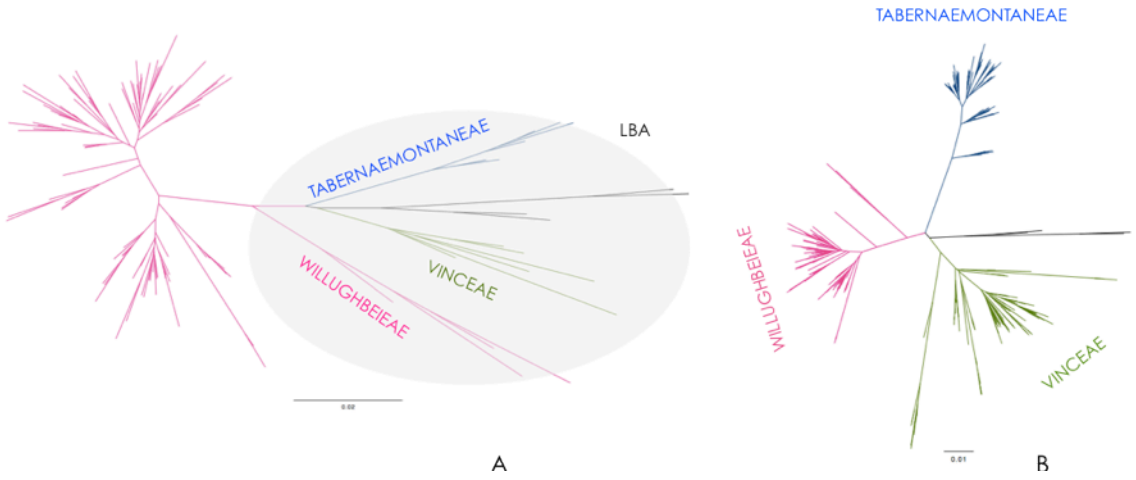


Fig. 7. Phylogram in radial tree layaut. A. Willughbeieae phylogram derived from Bayesian analysis in MrBayes, branches with possible long-branch attraction (LBA) in shadow. B. ViWiTa phylogram derived from Bayesian analysis in Beast.

Ttttt

Fig. 8. Reconstructed ancestral states of character 1 (Habit), over 100 trees, using parsimony criterion in Mesquite. White: tree; Blue: shrubs; Green: herbs; Black: lianas; Red: node ausent; Gray: equivocal reconstruction.

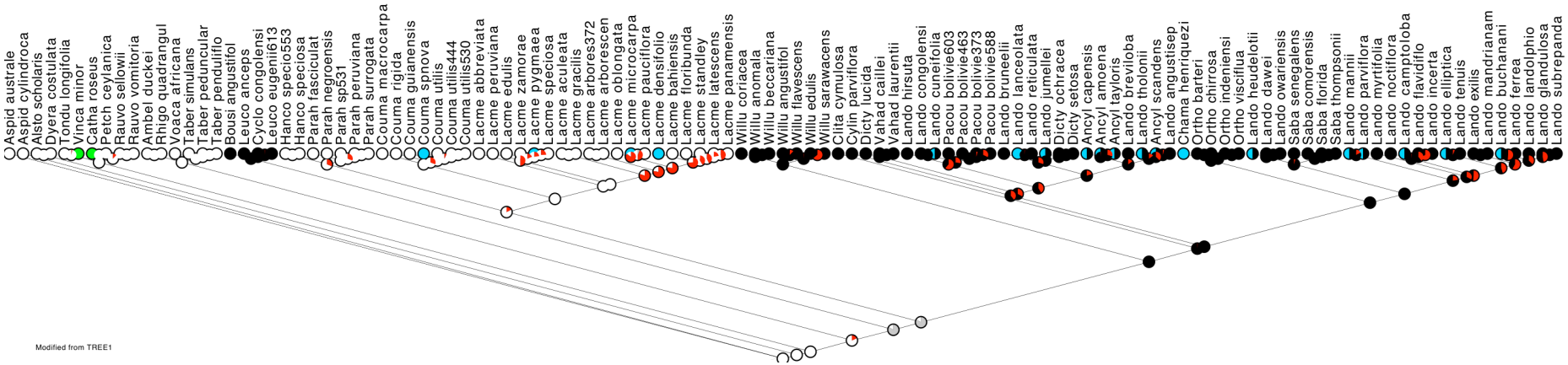


Fig. 9. Reconstructed ancestral states of character 8 (Tendrils), over 100 trees, using parsimony criterion in Mesquite. White: absent; Black: present; Red: node ausent; Gray: equivocal reconstruction.

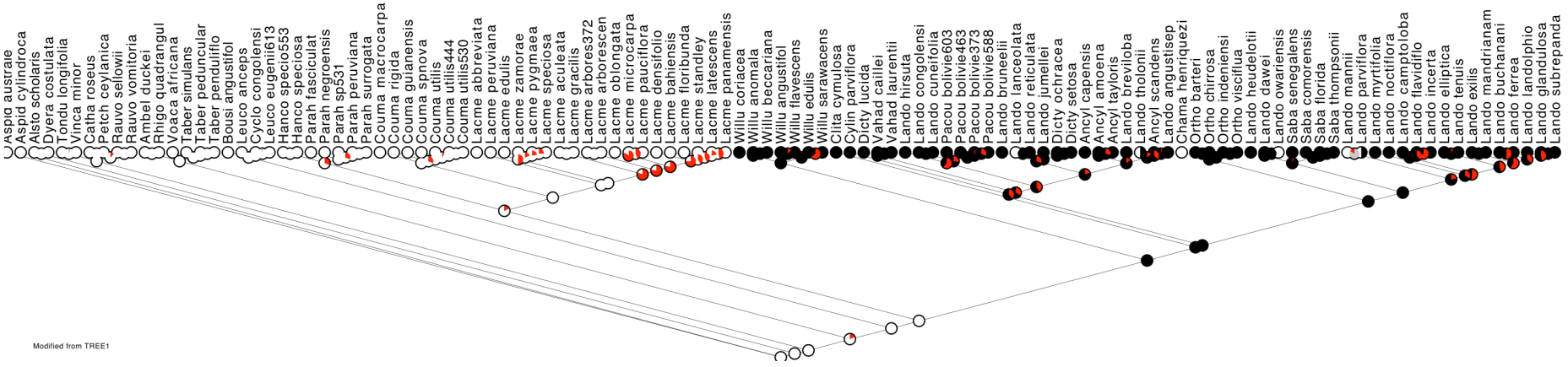


Fig. 10. Reconstructed ancestral states of character 10 (Inner surface of the calyx), over 100 trees, using parsimony criterion in Mesquite. White: naked; Green: with reduced standard colleters at the margim of sepals; Black: with standard colleters at the axils of the sepals; Red: node ausent; Gray: equivocal reconstruction.

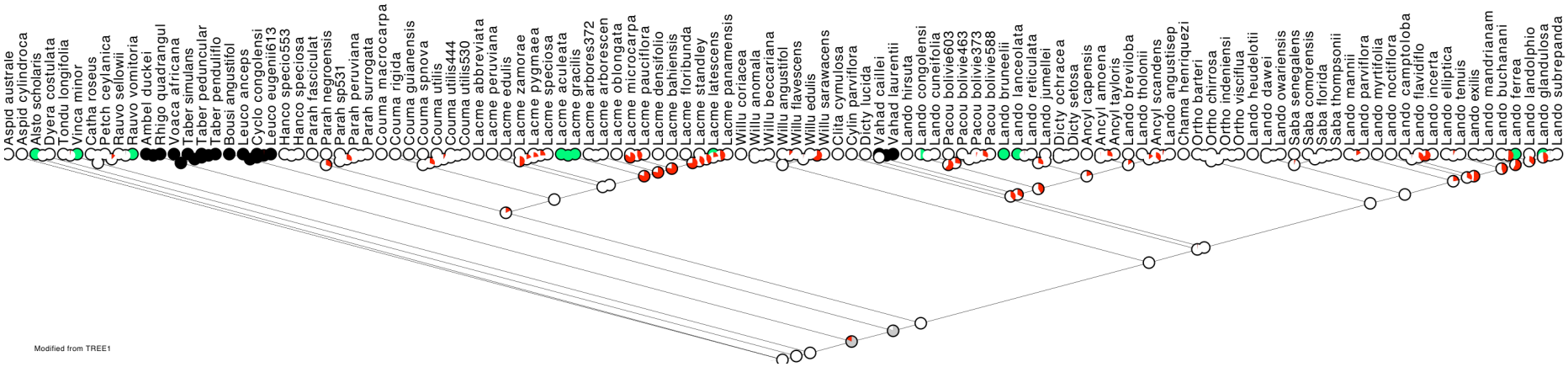


Fig. 11. Reconstructed ancestral states of character 18 (Style-head body apex), over 100 trees, using parsimony criterion in Mesquite. White: undifferentiated; Green: with un-lobed upper wreath; Black: with deeply 5- to 10-lobed upper crest; Red: node ausent; Gray: equivocal reconstruction.

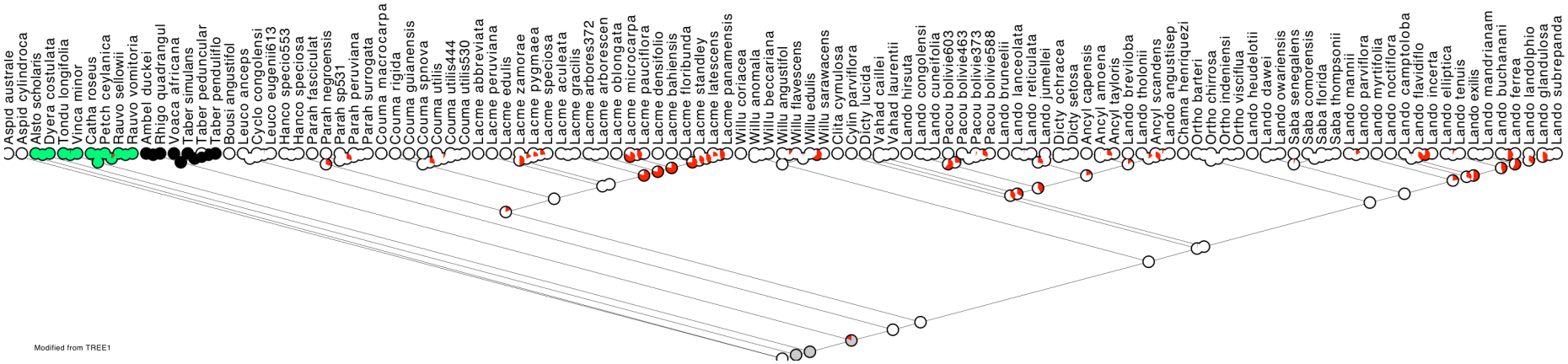


Fig. 12. Reconstructed ancestral states of character 19 (Style-head body base), over 100 trees, using parsimony criterion in Mesquite. White: undifferentiated; Green: with membranous collar; Black: with thickened flange; Red: node ausent; Gray: equivocal reconstruction.

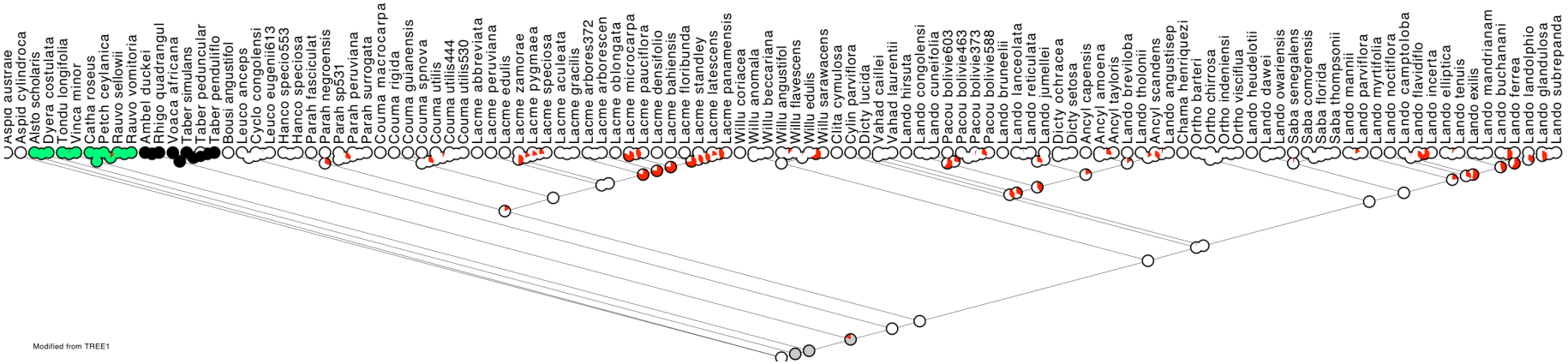


Fig. 13. Reconstructed ancestral states of character 23 (Ovary fusion), over 100 trees, using parsimony criterion in Mesquite. White: apocarpous; Black: syncarpous; Red: node ausent; Gray: equivocal reconstruction.

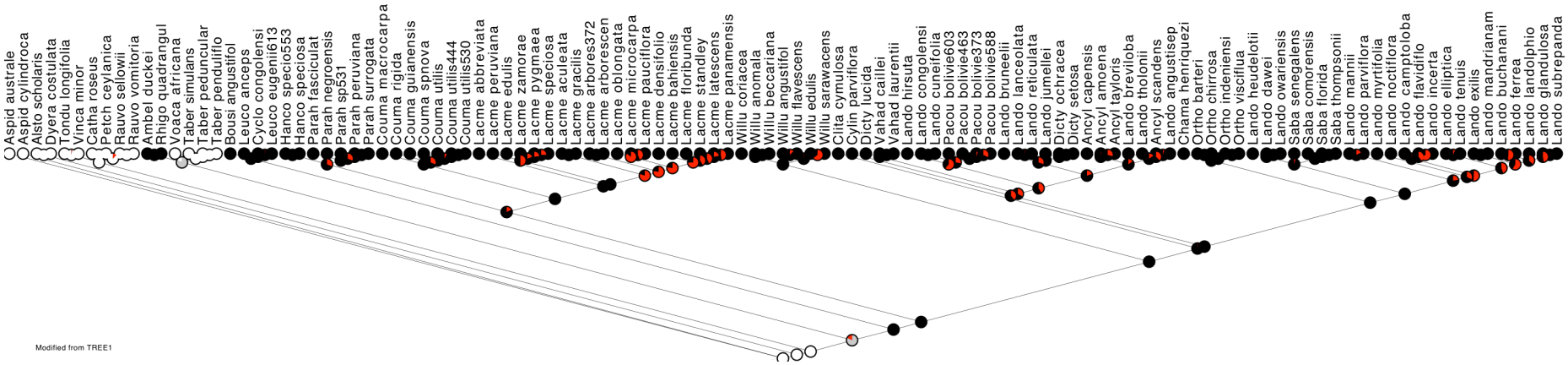


Fig. 14. Reconstructed ancestral states of character 24 (Ovary position), over 100 trees, using parsimony criterion in Mesquite. White: superior; Black: half-inferior; Red: node ausent; Gray: equivocal reconstruction.

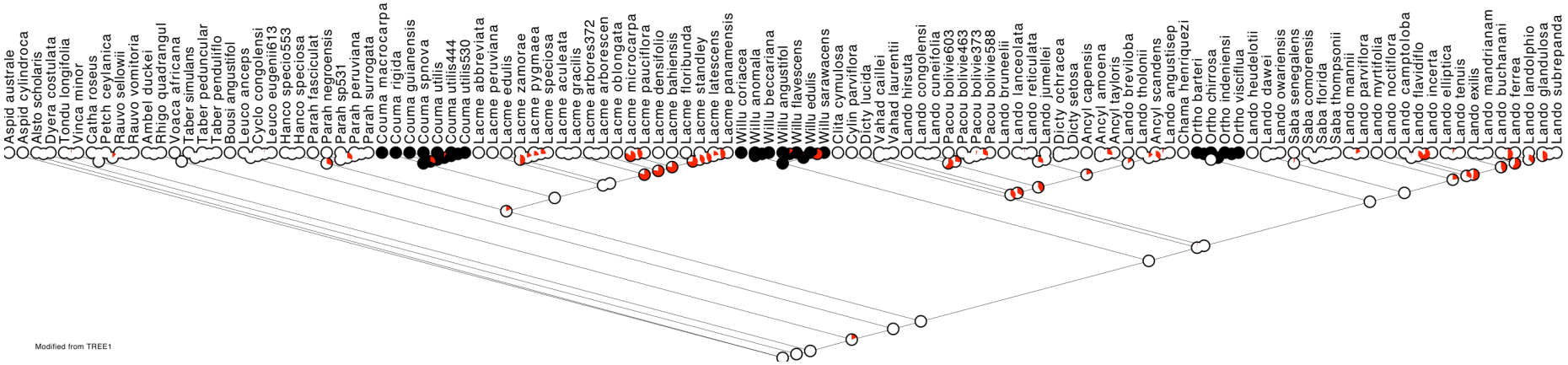


Fig. 15. Reconstructed ancestral states of character 25 (Ovary surface), over 100 trees, using parsimony criterion in Mesquite. White: smooth; Black: with trichomes; Red: node ausent; Gray: equivocal reconstruction.

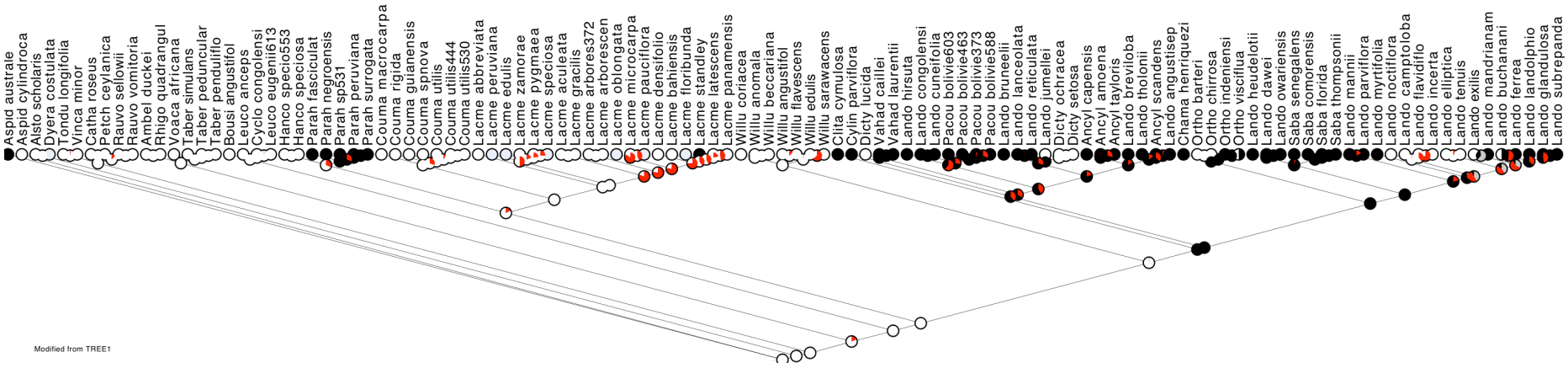


Fig. 16. Reconstructed ancestral states of character 26 (Ovary shape), over 100 trees, using parsimony criterion in Mesquite. White: cylindrical; Green: spherical to quadrangular; Black: cuneiform; Red: node ausent; Gray: equivocal reconstruction.

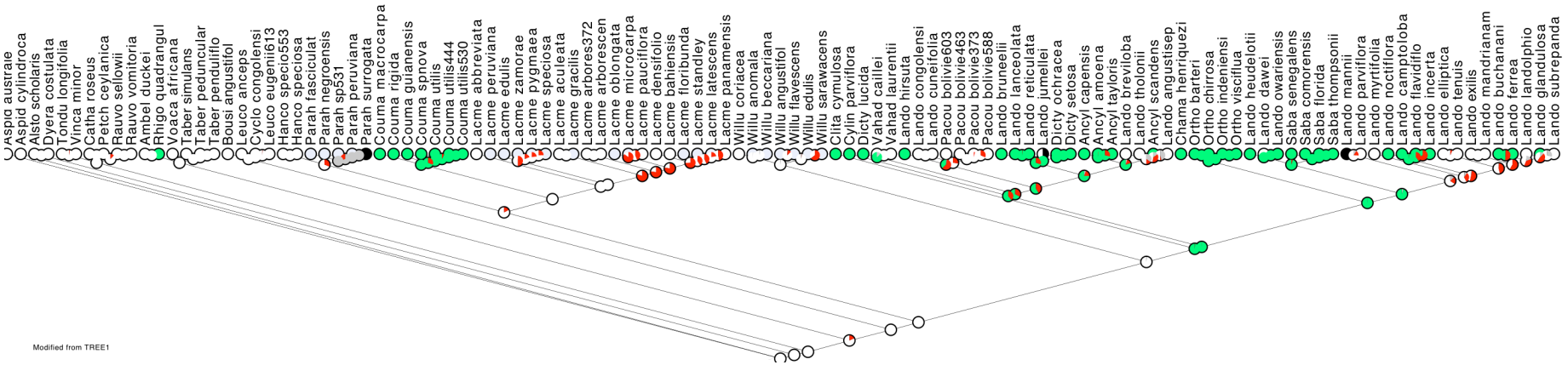


Fig. 17. Reconstructed ancestral states of character 28 (Ovules per carpel), over 100 trees, using parsimony criterion in Mesquite. White: 1-10; Black: numerous; Red: node ausent; Gray: equivocal reconstruction.

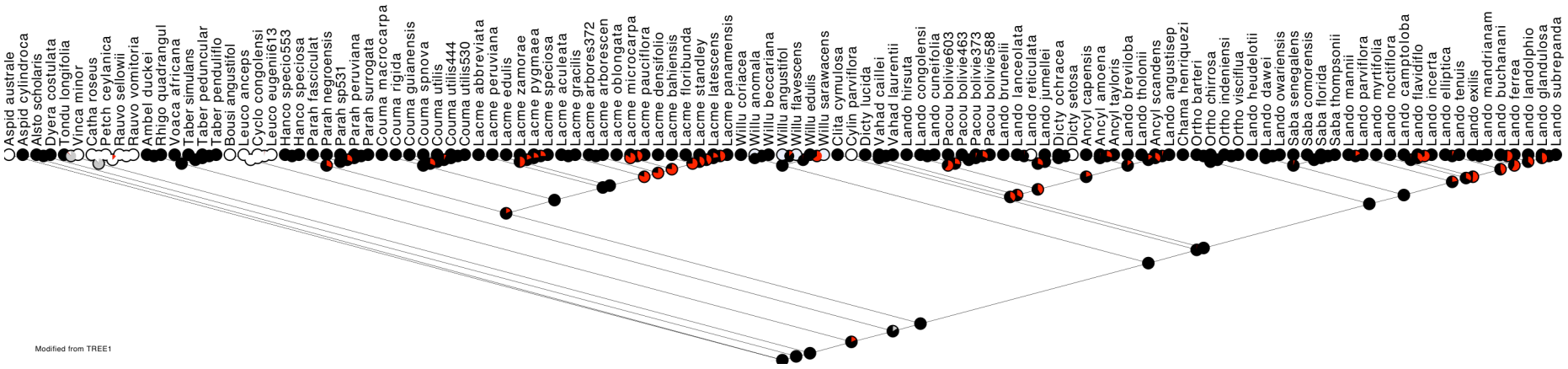


Fig. 18. Reconstructed ancestral states of character 29 (Placentation), over 100 trees, using parsimony criterion in Mesquite. White: marginal; Black: parietal; Red: node ausent; Gray: equivocal reconstruction.

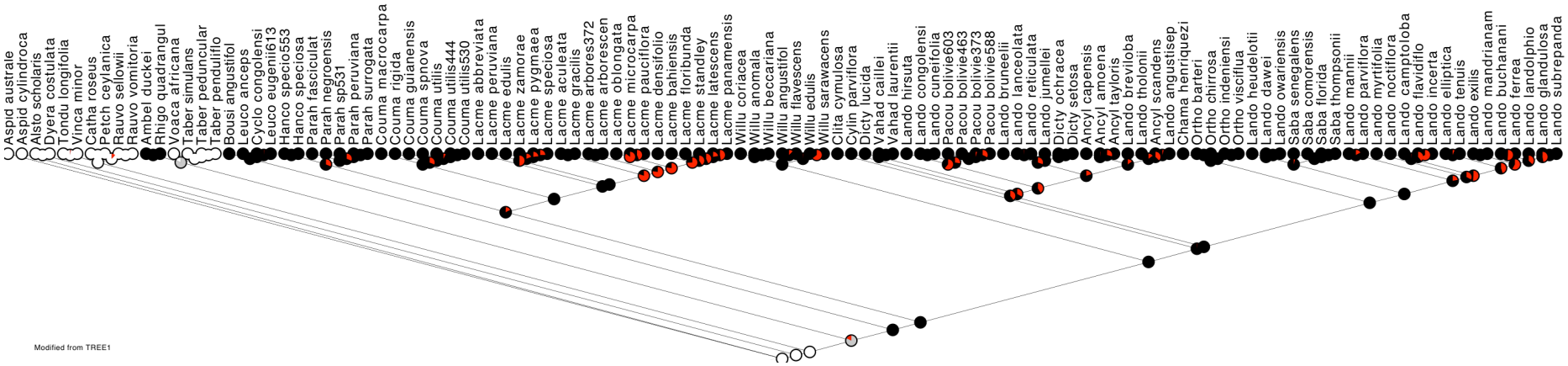


Fig. 19. Reconstructed ancestral states of character 30 (Number of locules), over 100 trees, using parsimony criterion in Mesquite. White: 1; Black: 2; Red: node ausent; Gray: equivocal reconstruction.

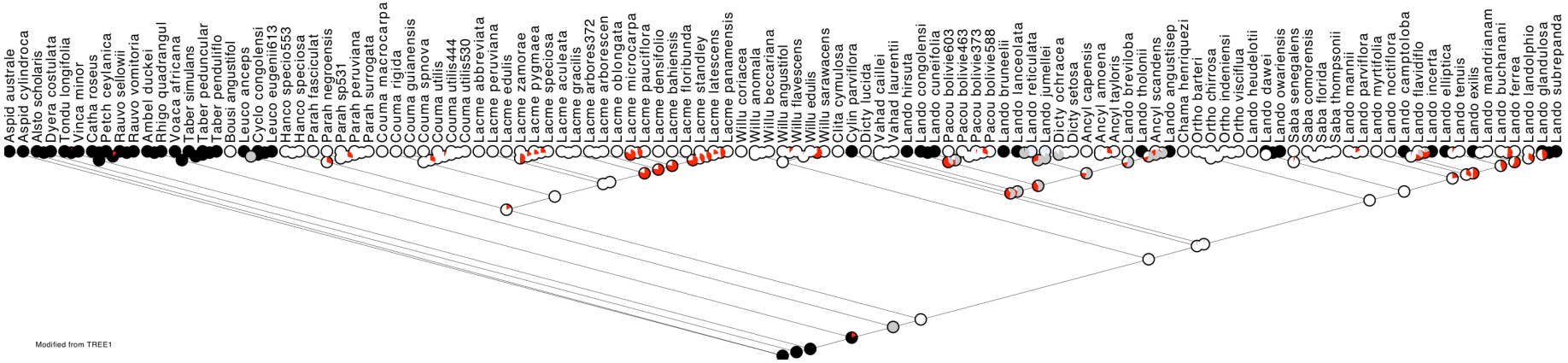


Fig. 20. Reconstructed ancestral states of character 31 (Fruit type), over 100 trees, using parsimony criterion in Mesquite.

White: follicular; Green: baccate; Black: drupaceous; Red: node ausent; Gray: equivocal reconstruction.

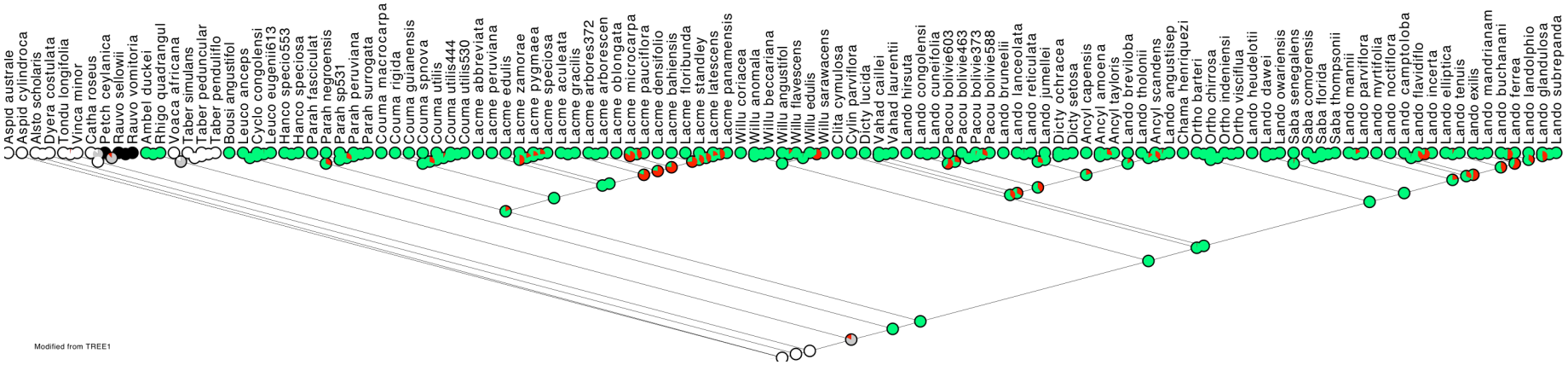
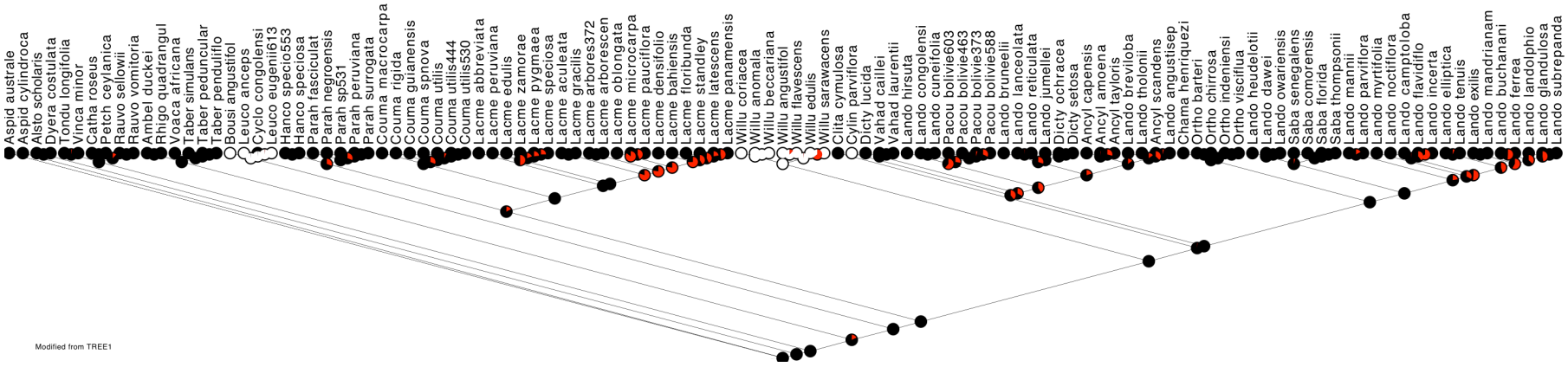


Fig. 21. Reconstructed ancestral states of character 37 (Endosperm), over 100 trees, using parsimony criterion in Mesquite.

White: absent or scanty; Black: present; Red: node ausent; Gray: equivocal reconstruction.



Appendix 1: Species names, voucher information and GenBank acession for sequences used in the molecular analyses (rpl16; *rps16; trnS-G; trnK; matK,* respectively) obtained from previous studies (Simões et al. 2007, 2010)

Alstonia scholaris (L.) R. Br.; Sabah; *S.J. Davies & al. SJD99150* (A); DQ660748; DQ660558; GU974062; DQ660680; Z70189. ***Aspidosperma australe*** Müll. Arg.; Brazil; *L.S. Kinoshita 02/110* (UEC); DQ660565; DQ660565; GU974064; DQ660687; DQ660502. ***Aspidosperma cylindrocarpon*** Müll. Arg.; Brazil, cult. University of Campinas; *A.O. Simões 1328* (UEC); DQ660756; DQ660566; GU974065; DQ660688; DQ660503. ***Catharanthus roseus*** (L.) G. Don cult., Brazil, University of Campinas; *A.O. Simões 1329* (UEC); DQ66076; DQ660570; - ; DQ660692; DQ660507. ***Couma guianensis*** Aubl.; French Guiana; *M.F. Prévost 3798* (CAY); DQ660765; DQ660575; GU974073; DQ660697; DQ660512. ***Dyera costulata*** Hook. f.; cult., Singapore, Bot. Gard. Singapore; *Ian Turner s.n.* (SING); DQ660768; DQ660578; - ; DQ660700; DQ660515. ***Hancornia speciosa*** Gomes; Brazil; *L.S. Kinoshita 02/101* (UEC); DQ660774; DQ660584; GU974075; DQ660705; DQ660519. ***Lacmellea aculeata*** (Ducke) Monach.; French Guiana; *M.F. Prévost 3803* (CAY, P, Z); DQ660779; DQ660589; GU974076; DQ660710; DQ660523. ***Parahancornia fasciculata*** (Poir.) Benoist; French Guiana; *M.F. Prévost 3801* (CAY, P, Z); DQ660787; DQ660597; GU974082; DQ660718; DQ660530. ***Rhigospira quadrangularis*** (Müll.Arg.) Miers: Brazil, *A.O. Simões 03/2008* (INPA, UEC); GU974178; GU973988; GU974084; GU974262; GU973904. ***Rauvolfia vomitoria*** Afzel.; cult. Brazil, University of Campinas; *I. Koch 871* (UEC); DQ660797; DQ660607; GU974083; DQ660728; DQ660538. ***Tabernaemontana simulans*** (J.F. Morales & Q. Jiménez) A.O. Simões

& M.E. Endress (**=*Stemmadenia simulans***); Costa Rica; *J.F. Morales 9823* (INB); GU974184; GU973994; GU974093; GU974268; GU973910. ***Saba comorensis*** (Bojer ex A. DC.) Pichon; cult. Belgium, Bot. Gard. Meise; *F. Billiet 3442* (BR); DQ660798; DQ660608; GU974085; DQ660729; DQ660539. ***Tabernaemontana peduncularis*** Wall.; Thailand, *D.J. Middleton 3368* (A); GU974229; GU974039; GU974142; GU974313; GU973955. ***Tabernaemontana penduliflora*** K. Schum.; cult. Netherlands, Wageningen Bot. Gard.; acc. number 75PT00851 (unvouchered); GU974230; GU974040; GU974143; GU974314; GU973956. ***Tonduzia longifolia*** (A. DC.) Markgr.; Mexico; *Munn-Estrada 2165* (MEXU); DQ660812; DQ660622; GU974161; DQ660742; DQ660552. ***Vinca minor*** L.; Switzerland, cult. Bot. Gard. Zurich; *A.O. Simões 1332* (Z); DQ660813; DQ660623; GU974162; DQ660743; DQ660553. ***Voacanga africana*** Stapf; cult. Belgium, Bot. Gard. Meise; *F. Billiet S2276* (BR); GU974248; GU974058; GU974163; GU974332; GU973974. ***Willughbeia angustifolia*** (Miq.) Markgraf; Brunei; *D.J. Middleton & al. 727* (A); DQ660814; DQ660624; GU974167; DQ660744; DQ660554.

Appendix 2: Taxon names and voucher information.

Taxon	Country, Locality	Collector	Number	Herbarium	Sample
Ancylobothrys amoena Hua	Guine, Nzérékoré	*C.C.H. Jongkind*	*7397*	BR	exsicate
Ancylobothrys capensis (Oliv.) Pichon	South Africa, Pretoria	*H. Breyne*	*5265*	BR	exsicate
Ancylobothrys scandens (Schumach.) Pichon	Congo	*F. Dowsett-Lemaire*	*1400*	BR	exsicate
Ancylobothrys tayloris (Stapf) Pichon	Tanzania	*Research Programme*	*2220*	BR	exsicate
Bousigonia angustifolia Pierre ex Spire	Thailand	*P.K. Harder*	*4763*	A	exsicate
Chamaeclitandra henriqueziana (Hallier f.) Pichon	Congo	*M. Schaites*	*3117*	BR	exsicate
Clitandra cymulosa Benth.	Gabon	*A.J.M. Leeuwenberg*	*13585*	WAG	exsicate
Couma guianensis Aubl.	French Guiana	*M.F. Prévost*	*3798*	CAY	exsicate
Couma macrocarpa Barb. Rodr.	Costa Rica	*J.F. Morales*	*s/n*	INB, UEC	silica
Couma rigida Müll. Arg.	Brazil, Bahia	*?*	*?*	*?*	silica
Couma utilis (Mart.) Müll. Arg.	Brazil, Amazonas	*A.O. Simões & al.*	*?*	*?*	silica
Couma utilis (Mart.) Müll. Arg.	Brazil, Amazonas	*F.A.C*	*2017*	INPA	silica
Couma utilis (Mart.) Müll. Arg.	Brazil, Roraima	*M. Hopkins*	*2021*	INPA	silica
Cyclocotyla congolensis Stapf	Gabon	*Caballe*	*384*	WAG	exsicate
Cylindropsis parvifolia Pierre	Gabon	*A.J.M. Leeuwenberg*	*13563*	BR, WAG	exsicate
Dictyophleba lucida (K. Schum.) Pierre	Malawi	*F. Dowsett-Lemaire*	*692*	BR	exsicate
Dictyophleba ochracea (K . Schum. ex Hallier f.) Pichon	Gabon	*C.C.H. Jongkind*	*1338*	MO	exsicate
Dictyophleba setosa B. de Hoogh	Equatorial Guinea	*Carvalho*	*5901*	BR	exsicate
Hancornia speciosa Gomes	Brazil	*L.S. Kinoshita*	*02/101*	UEC	silica
Hancornia speciosa var pubescens (Nees & Mart.) Müll. Arg.	Brazil, Distrito Federa	*Alencar, M.E.*	*471*	UEC	exsicate
Lacmellea abbreviata J.F. Morales	Colombia	*J. Espina & F. García*	*1528*	INB	silica
Lacmellea aculeata (Ducke) Monach.	French Guiana	*M.F. Prévost*	*3803*	CAY	exsicate
Lacmellea arborescens (Müll. Arg.) Markgr.(372)	Brazil, Amazonas	*A.O. Simões & al.*	*s/n*	INPA, UEC	silica
Lacmellea arborescens (Müll. Arg.) Markgr.	Brazil, Amazonas	*A.O. Simões & al.*	*?*	*?*	silica
Lacmellea bahiensis J.F. Morales	Brazil	*S.C. Sant'Ana*	*880*	NY	exsicate
Lacmellea densifoliata (Ducke) Markgr.	Brazil, Pará	*G.L. Sobel*	*4583A*	INB, MG	exsicate
Lacmellea edulis H. Karst.	*?*	*?*	*?*	*?*	
Lacmellea floribunda (Poepp.) Benth. & Hook. f.	Ecuador	*J.L. Clarck & al.*	*4899*	MO, Z	exsicate
Lacmellea gracilis (Müll. Arg.) Markgr.	Brazil, Amazonas	*A.O. Simões & al.*	*?*	*?*	silica
Lacmellea latescens (Kuhlm.) Markgrf.	Brazil, Cult. Jardim Botânico do Rio de Janeiro	-	-	-	silica
Lacmellea microcarpa (Müll. Arg.) Markgr.	Brazil, Amazonas	*R. Morokawa*	*273*	INPA	silica
Lacmellea oblongata Markgr.	Peru	*A. Gentry & D. Alfero*	*37944*	INB, MO	exsicate

Appendix 2: Continued.

Taxon	Country	Collector	Number	Herbarium	
Lacmellea panamensis ((Woodson) Markgrf.	Costa Rica	*J.F. Morales*	*s/n*	INB, UEC	silica
Lacmellea pauciflora (Kuhlm.) Markgr.	Brazil, Espirito Santo	*L.Kollmann*	*5811*	UEC	exsicate
Lacmellea peruviana (Van Heurck & Müll. Arg.) Markgr.	Peru, Ucayali	*J. Graham*	*534*	INPA	exsicate
Lacmellea pygmaea Monach.	Venezuela	*P.E. Berry*	*5501*	MO, Z	exsicate
Lacmellea speciosa Woodson	Costa Rica, San José	*J.F. Morales*	*4357*	INB	silica
Lacmellea standley (Woodson) Monach.	Belize, Coyo Distrit	*C. Whiteford & Pacheco*	*9416*	INB	exsicate
Lacmellea zamorae J.F. Morales	Costa Rica	*J.F. Morales*	*s/n*	INB, UEC	silica
Landolphia angustisepala Pichon	Gabon	*J.J.F.E. de Wilde*	*409*	RB	exsicate
Landolphia breviloba J.G.M. Pers.	Gabon	*J.J.F.E. de Wilde*	*230*	BR, WAG	exsicate
Landolphia bruneelii (De Wild.) Pichon	Gabon	*J.J.F.E. de Wilde*	*236*	BR	exsicate
Landolphia buchananii (Hallier f.) Stapf	Tanzania	*J. Lovett*	*3751*	BR, MO	exsicate
Landolphia camptoloba (K. Schum.) Pichon	Congo, Bombo Lumene	*Nsimundele*	*1890*	BR	exsicate
Landolphia congolensis (Stapf) Pichon	Equatorial Guinea	*Carvalho*	*5979*	BR	exsicate
Landolphia cuneifolia Pichon	Congo, Kolwezi	*F. Malaisse*	*14152*	BR	exsicate
Landolphia dawei Stapf	Belgium, cult. Meise Botanical Garden	*BR (19514750)*	*s/n*	BR	silica
Landolphia elliptica Lassia	Madagascar, Fort Dauphin	*A.J.M. Leeuwenberg*	*14200*	BR, WAG	exsicate
Landolphia exilis Jum. & H. Perrier	Madagascar	*R. Randrianaivo*	*1219*	MO	exsicate
Landolphia ferrea J.G.M. Pers.	Gabon, Sindora	*A.J.M. Leeuwenberg*	*13615*	BR, LBV	exsicate
Landolphia flavidiflora (K. Schum.) J.G.M. Pers.	Cameroon, Kribi	*J.J.J. Bos*	*4205*	BR	exsicate
Landolphia glandulosa (Pellegr.) Pichon	Equatorial Guinea	*Conulho*	*5240*	BR	exsicate
Landolphia heudelotii A. DC.	Belgium, cult. Meise Botanical Garden	*BR (19773111)*		BR	silica
Landolphia hirsuta (Hua) Pichon	Equatorial Guinea	*O.O. Hulu*	*382*	BR	exsicate
Landolphia incerta (K. Schum.) J.G.M. Pers.	Belgium, cult. Meise Botanical Garden	*BR(19850056)*	-	BR	silica
Landolphia jumelleii (Pierre ex Jum.) Pichon	Cameroon	*A.J.M. Leeuwenberg*	*9709*	BR, WAG	exsicate
Landolphia lanceolata (K. Schum.) Pichon	Congo	*J. Lejoly*	*82/744*	Z	exsicate
Landolphia landolphioides (Hallier f.) A. Chev.	Cameroon	*G.J.H. Amshoff*	*2629*	Z	exsicate
Landolphia mandrianambo Pierre	Madagascar	*A.J.M. Leeuwenberg*	*14404*	BR, WAG	exsicate
Landolphia mannii Dyer	Congo	*Amsini*	*39*	BR	exsicate
Landolphia myrtifolia (Poir.) Markgr.	Madagascar	*F. Barthelat*	*402*	MO	exsicate
Landolphia noctiflora J.G.M. Pers.	Gabon	*Peerson*	*734*	BR	exsicate
Landolphia owariensis P. Beauv.	Belgium, cult. Meise Botanical Garden	*BR(PP18)*	-	BR	silica
Landolphia parvifolia K. Schum.	Zambia	*D.K. Hurder*	*3072*	BR, MO	exsicate
Landolphia reticulata Hallier f.	Gabon	*J.L.C.H. Valkenberg*	*2546*	BR	exsicate

Appendix 2: Continued.

Taxon	Country	Collector	Number	Herbarium	
Landolphia subrepanda (K. Schum.) Pichon	Equatorial Guinea	*Carvalho*	*5388*	BR	exsicate
Landolphia tenuis Jum.	Madagascar	*A.J.M. Leeuwenberg*	*14362*	BR, WAG	exsicate
Landolphia thollonii Dewèvre	Belgium, cult. Meise Botanical Garden	*BR (19391928)*	-	BR	silica
Leuconotis anceps Jack.	Indonesia, East Kalimantan	*A.J.M. Leeuwenberg*	*13028*	WAG	exsicate
Leuconotis eugeniifolia (Wall. Ex G. Don) A. DC.	Thailand	*Sidiyasa & Aritin*	*2034*	A-GH	exsicate
Orthopichonia barteri (Stapf) H. Huber	Gabon	*G. McPherson*	*16118*	MO	exsicate
Orthopichonia cirrhosa (Radlk.) H. Huber	Cameroon	*J.F. de Wilde*	*2567*	WAG	exsicate
Orthopichonia indeniensis (A. Chev.) H. Huber	Nigeria	*P.A. Talbot*	*s/n*	WAG	exsicate
Orthopichonia visciflua (K. Schum. ex Hallier f.) Vonk	Equatorial Guinea	*Carvalho*	*5976*	BR	exsicate
Pacouria boliviensis (Markgr.) A. Chev.	Bolivia, Santa Cruz	*R. Frey & M. Lewis*	*706*	Z	exsicate
Pacouria boliviensis (Markgr.) A. Chev.	Brazil, Minas Gerais	*J.A. Lombardi*	*3672*	BHCB, INB	exsicate
Pacouria boliviensis (Markgr.) A. Chev.	Peru	*R. Vasquez*	*21684*	MO	exsicate
Pacouria boliviensis (Markgr.) A. Chev.	Brazil, Amazonas	*A. Quinet*	*1293*	RB	exsicate
Parahancornia fasciculata (Poir.) Benoist	French Guiana	*M.F. Frost*	*3801*	Z	exsicate
Parahancornia negroensis Monach.	Brazil, Amazonas	*ABJ*	*890*	INPA	silica
Parahancornia peruviana Monach.	Peru	*R. Vasquez*	*14222*	MO	exsicate
Parahancornia sp	Brazil, Roraima	*R. Morokawa*	*287*	INPA	silica
Parahancornia surrogata Zarucchi	Colombia	*D. Restrepo*	*337*	MO	exsicate
Saba comorensis (Bojer ex A. DC.) Pichon	Belgium, cult. Meise Botanical Garden	*F. Billiet*	*3442*	BR	silica
Saba comorensis (Bojer ex A. DC.) Pichon	Nigeria	*B.O. Daramola*	*515*	MO	exsicate
Saba senegalensis (A. DC.) Pichon	Mali	*H. Breyne*	*6190*	BR	exsicate
Saba thompsonii (A. Chev.) Pichon	Benin	*V. Adjakidje*	*3757*	MO	exsicate
Vahadenia caillei (A. Chev.) Stapf ex Hutch. & Dalziel	Liberia	*van Meer*	*225*	BR, WAG	exsicate
Vahadenia laurentii (De Wild.) Stapf	Congo	*R. M'Boungou*	*457*	MO	exsicate
Willughbeia angustifolia (Miq.) Markgr.	Brunei	*D.J. Middleton &al.*	*727*	A	exsicate
Willughbeia anomala Markgr.	Brunei, Belait, Lumut	*M. Salleh &al.*	*15425*	L	exsicate
Willughbeia beccariana (Kuntze ex Pierre) K. Schum.	Brunei, Simpang	*K.M. Wong & S.E. Siou*	*WKM557*	L	exsicate
Willughbeia coriacea Wall.	Malaysia	*M. Eja*	*s/n*	MO	exsicate
Willughbeia edulis Roxb.	?	?	?	?	exsicate
Willughbeia flavescens Dyer ex Hook. f.	Malaysia, Negeri Semblia	*E.G.*	*1967*	L	exsicate
Willughbeia sarawacensis (Pierre) K. Schum.	Indonesia, East Kalimantan	*M. Koizumi & Lalo*	*8112*	L	exsicate

Appendix 3. Characters and character states for the morphological matrix used in the cladistic analyses.

1. Habit: trees (0), shrubs (1), herbs (2), lianas (3). **2. Phyllotaxis**: alternate (0), opposite (1), whorled (2). **3. Interpetiolar stipels**: absent (0), present (1). **4. Dots on leaves**: absent (0), present (1). **5. Inflorescence position**: terminal (0), axyllary (1). **6. Inflorescence organization**: lax (0), congested (1). **7. Inflorescence tendril**-like: inapplicable (-), no (0), yes (1). **8. Tendrils**: absent (0), present (1). **9. Flower simmetry**: actinomorphic (0), zygomorphic (1). **10. Inner surface of the calyx**: naked (0), with reduced standard colleters at the margin of the sepals (1), with standard colleters at the axils of the sepals (2). **11. Corolla color**: white (0), pink to lilac (1), yellow (2). **12. Proportion between corola lobes and tube**: 1:1 (0), 1:2 (1), 1:3 (2), 3:1 (3). **13. Position of the anther tips at the corolla tube**: at the corolla throat (0), middle portion of the corolla tube (1). **14. Corolla lobes margin**: non-ciliate (0), ciliate (1). 15. **Anthers**: non-carenate (0), carenate (1). **16. Style-head body shape**: cylindrical to shperical (0), fusiform (1). **17. Calyx lobes**: equal (0), unequal (1). **18. Style-head body apex**: undifferentiated (0), with un-lobed upper wreath (1), with deeply 5- to 10-lobed upper crest (2). **19. Style-head body base**: undifferentiated (0), with membranous collar (1), with thickened flange (2). **20. Style-head apical appendages size**: smaller than the main body (0), equaling or surpassing the main body (1). **21. Style**: inconspicuous (0), conspicuous (1). **22. Style surface**: smooth (0), with trichomes (1). **23. Ovary fusion**: apocarpous (0), hemisyncarpous (1), syncarpous (2). **24. Ovary position**: superior (0), half-inferior (1). **25. Ovary surface**: smooth (0), with trichomes (1). **26. Ovary shape**: cylindrical

(0), spherical to quadrangular (1), cuneiform (2). **27. Nectary**: absent or inconspicuous (0), forming a disk around the ovary base (1), 2 lobes alternating with the carpels (2). **28. Ovules per carpel**: 1-10 (0), 11-numerous (1). **29. Placentation**: marginal (0), axyle (1), parietal (2). **30. Number of locules**: 1 (0), 2 (1). **31. Fruit type**: follicular (0), baccate (1), drupaceous (2). **32. Indument on fruit**: absent (0), present (1). **33. Fruit surface**: smooth (0), lenticellate (1), muricate (2). **34. Mesocarp consistency**: fleshy or spongy (0), fibrous (1), dry or woody (2). **35. Pericarp with sclerified layer**: no (0), yes (1). **36. Sclerified base of style present in ripe fruits**: no (0), yes (1). **37. Endosperm**: absent or scanty (0), present (1). **38. Number of functional apertures of pollen grains**: 3-4 (0), 1-2 (1). **39. Corolla merosity**: 5-merous (0), 4-merous (1). **40. Spines on trunk**: absent (0), present (1).

Appendix 4. Morphological matrix coded. Polymorphic states are indicated by numbers united by &, missing data = ?, unapplied = - .

	Character states																																							
	0									1										2										3										4
Taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**
Aspid australe	0	0	0	0	0	0	-	0	0	0	0	2	0	1	0	0	0	0	0	?	1	0	0	0	1	0	0	0	0	1	0	0	1	2	0	0	1	0	0	0
Aspid cylindroca	0	0	0	0	0	0	-	0	0	0	0	3	0	?	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	2	0	0	1	0	0
Alsto scholaris	0	2	0	0	0	0	-	0	0	1	0	2	0	?	0	0	?	1	1	1	1	0	0	0	0	0	0	1	0	1	0	0	1	2	0	0	1	0	?	0
Dyera costulata	0	2	0	0	0	0	-	0	0	0	0	0	?	?	0	?	?	1	1	?	?	0	0	0	?	0	0	1	0	1	0	0	1	2	0	0	1	0	?	0
Ambel duckei	0	1	0	0	1	1	-	0	0	2	0	0	1	0	0	0	0	2	2	1	1	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1	0	0	0
Rhigo quadrangul	0	1	0	0	0	0	-	0	0	2	0	0	1	0	0	0	0	2	2	1	1	0	2	0	0	1	0	1	2	1	1	0	0	0	0	0	1	0	0	0
Taber peduncular	0	1	0	0	0	0	-	0	0	2	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
Taber penduliflo	0	1	0	0	0	0	-	0	0	2	0	3	0	0	0	0	0	2	2	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0
Taber simulans	0	1	0	0	0	1	-	0	0	2	2	2	1	0	0	0	0	2	2	0	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
Voaca africana	0	1	0	0	0	0	-	0	0	2	2	3	0	0	0	?	0	2	2	?	1	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0	0	0
Catha roseus	2	1	0	0	0	0	-	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	2	0	0	1	0	0	1	2	0	0	1	0	0	0
Petch ceylanica	0	2	0	0	0	0	-	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	1	0	0	0
Rauvo sellowii	0	2	0	0	0	0	-	0	0	0	0	2	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	1	0	0	0
Rauvo vomitoria	0	2	0	0	0	0	-	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	1	2	0	0	0	0	0	1	0	0	0
Tondu longifolia	0	2	0	0	0	0	-	0	0	0	0	0	?	?	0	?	?	1	1	1	1	0	0	0	0	0	0	1	0	1	0	0	1	2	0	0	1	0	0	0
Vinca minor	2	1	0	0	1	1	-	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	2	0	0	1	0	0	1	2	0	0	1	0	0	0
Ancyl amoena	1&3	1	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	0	1	1	0	2	0	1	1	0	1	2	0	1	1	0	0	0	0	1	0	0	0
Ancyl capensis	1&3	1	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0	0	0	1	1	0	2	0	1	1	0	1	2	0	1	1	0	0	0	0	1	0	0	0
Ancyl scandens	1&3	1	0	1	0	0	1	1	0	0	0	2	1	1	1	0	0	0	0	1	1	0	2	0	1	1	0	1	2	0	1	1	0	0	0	0	1	0	0	0
Ancyl tayloris	1&3	1	0	1	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	0	2	0	1	1	0	1	2	0	1	1	0	0	0	0	1	0	0	0
Bousi angustifol	3	1	0	1	1	0	-	0	0	2	0	1	?	0	?	0	0	0	0	1	1	0	2	0	0	0	1	0	2	0	1	0	0	0	0	0	0	0	0	0
Chama henriquezi	1	1	0	1	0&1	1	-	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Clita cymulosa	3	1	0	0	1	0&1	0	1	0	0	0	2	1	1	0	1	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Couma spnova	1	2	0	1	0	1	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Couma guianensis	0	2	0	0	0	0	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Couma macrocarpa	0	2	0	0	0	0	-	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Couma rigida	0	2	0	1	0	1	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0

Appendix 4. Continued

	Character states																																						
	0									1										2										3									4
Taxon	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
Couma utilis	0	2	0	0	0	0	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Couma utilis444	0	2	0	0	0	0	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Couma utilis530	0	2	0	0	0	0	-	0	0	0	1	0	1	1	0	0	0	0	0	1	1	0	2	1	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Cyclo congolensi	3	1	0	1	0	0	-	0	0	2	0	1	0	0	0	0	1	0	0	1	0	0	2	0	0	0	1	0	2	1	1	0	0	0	0	0	0	0	0	0
Cylin parviflora	3	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	?	2	0	1	1	0	0	2	1	1	0	1	0	1	0	1	0	0	0
Dicty lucida	3	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	0	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Dicty ochracea	3	1	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	0	0	1	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Dicty setosa	3	1	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	0	0	1	0	0	2	0	1	0	0	0	0	0	1	0	0	0
Hanco specio553	0	1	0	0	0	1	-	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	2	0	0	0	0	1	2	0	1	0&1	0	0	0	0	1	0	0	0
Hanco speciosa	0	1	0	0	0	1	-	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	2	0	0	0	0	1	2	0	1	0&1	0	0	0	0	1	0	0	0
Lacme aculeata	0	1	0	0	1	1	-	0	1	1	0	2	1	1	0	2	0	0	0	1	1	0	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme arbores372	0	1	0	0	1	1	-	0	1	1	0	2	?	0&1	?	2	0	0	0	?	1	0	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme arborescen	0	1	0	1	1	1	-	0	1	0	0	2	?	0&1	0	2	0	0	0	?	1	0	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme bahiensis	0	1	0	0	1	1	-	0	1	0	0	2	0	?	0	2	0	0	0	1	1	?	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme abbreviata	0	1	0	0&1	1	1	-	0	0	0	0	2	0	0	0	2	0	0	0	?	0	?	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme densifolio	1	1	0	0&1	1	1	-	0	0	0	0	2	?	1	0	2	0	0	0	?	0	?	2	0	0	0	0	1	2	0	1	?	0	0	0	1	1	0	0	?
Lacme edulis	0	1	0	1	1	1	-	0	1	0	0	2	?	1	0	2	0	0	0	?	?	?	2	0	?	?	?	0	1	2	0	1	?	0	0	0	1	1	0	0	1
Lacme floribunda	0	1	0	0	1	1	-	0	1	0	0	?	?	?	?	2	0	0	0	?	?	?	2	0	?	?	?	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme gracilis	0	1	0	0	1	1	-	0	1	1	0	2	0	?	?	2	0	0	0	?	?	0	2	0	0	?	?	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme latescens	0	1	0	1	1	1	-	0	1	1	0	2	0	0	0	2	0	0	0	?	1	0	2	0	0	?	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme microcarpa	1	1	0	1	1	1	-	0	1	0	0	2	0	?	0	2	0	0	0	?	1	0	2	0	0	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme oblongata	0	1	0	0	1	1	-	0	1	0	0	2	0	0	0	2	0	0	0	?	?	?	2	0	?	?	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme panamensis	0	1	0	0	1	1	-	0	1	0	0	2	0	0	0	2	0	0	0	0	?	0	2	0	0	0	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme pauciflora	0	1	0	0	1	1	-	0	1	0	0	2	0	0	0	2	0	0	0	?	1	1	2	0	?	0	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme peruviana	0	1	0	1	1	1	-	0	1	0	0	?	?	?	0	2	0	0	0	?	?	?	2	0	?	?	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme pygmaea	1	1	0	0	1	1	-	0	1	0	0	2	0	0	0	2	0	0	0	0	0	?	2	0	0	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0

Appendix 4. Continued

	Character states																																						
	0									1										2									3										4
Taxon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**
Lacme speciosa	0	1	0	1	1	1	-	0	1	0	0	?	?	0	0	2	0	0	0	?	?	?	2	0	?	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lacme standley	0	1	0	0	1	1	-	0	1	0	0	3	1	0	0	2	0	0	0	0	1	1	2	0	1	?	0	1	2	0	1	0	0	0	0	1	1	0	0	1
Lacme zamorae	0	1	0	0&1	1	1	-	0	1	0	0	1	0	0	0	2	0	0	0	?	?	0	2	0	0	?	0	1	2	0	1	0	0	0	0	1	1	0	0	0
Lando angustisep	3	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	1	2	0	1	0	0	1	2	1	1	?	?	0	1	0	1	0	0	0
Lando breviloba	3	1	0	1	1	1	0	1	0	0	0	2	0	1	0	0	0	0	0	1	1	1	2	0	1	0	0	1	2	0	1	0	?	0	0	0	1	0	0	0
Lando bruneelii	3	1	0	1	1	1	0&1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	1	2	0	1	1	0	1	2	1	1	?	?	0	0	0	1	0	0	0
Lando buchanani	1&3	1	0	0	0	1	0&1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	2	0	0&1	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando camptoloba	1&3	1	0	0	0	0	0&1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	2	0	0	1	0	1	2	1	1	1	1	0	1	0	1	0	0	0
Lando congolensi	3	1	0	1	1	0	0	1	0	0&1	0	0	0	1	0	0	1	0	0	1	1	1	2	0	1	0	0	1	2	1	1	0	1	0	0	0	1	0	0	0
Lando cuneifolia	1&3	1	0	1	1	0	0	1	0	0	2	0	0	1	0	0	1	0	0	1	1	1	2	0	1	0	0	1	2	1	1	0	1	0	0	0	1	0	0	0
Lando dawei	3	1	0	1	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	1	1	0	1	0	1	0	1	0	0	0
Lando elliptica	1&3	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	2	0	0	0	0	1	2	1	1	0	1	0	1	0	1	0	0	0
Lando exilis	3	1	0	1	0&1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	0	0	0	1	2	1	1	0	1	0	1	0	1	0	0	0
Lando ferrea	3	1	0	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	0	0	1	0	0	0
Lando flavidiflo	3	1	0	1	0&1	0	0&1	1	0	0	2	3	0	1	0	0	1	0	0	1	1	0	2	0	0	1	0	1	2	0	1	?	?	0	?	0	1	0	0	0
Lando glandulosa	3	1	0	1	1	1	0	1	0	1	0	3	0	1	0	0	1	0	0	0	1	0	2	0	1	1	0	1	2	1	1	0	0	0	0	0	1	0	0	0
Lando heudelotii	1&3	1	0	1	0	1	0&1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando hirsuta	3	1	0	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	2	0	1	1	0	1	2	1	1	0	0	0	0	0	1	0	0	0
Lando incerta	3	1	0	1	0&1	0	0&1	1	0	0	0	3	0	1	0	0	0	0	0	1	0	0	2	0	0	1	0	1	2	1	1	0	1	0	0	0	1	0	0	0
Lando jumellei	1&3	1	0	0	1	1	0	1	0	0	2	0	0	?	0	0	1	0	0	1	1	0	2	0	0	0&2	0	1	2	?	1	0	1	0	0	0	1	0	0	0
Lando lanceolata	1	1	0	1	0	0	-	0	0	1	2	0	0	1	0	0	1	0	0	1	1	1	2	0	1	1	0	1	2	1	1	0	1	0	0	0	1	0	0	0
Lando landolphio	3	1	0	1	0&1	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando mandrianam	3	1	0	1	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando mannii	1&3	1	0	0	0	1	-	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	1	2	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando myrtifolia	3	1	0	0	0&1	1	0	1	0	0	0	1	1	1	0	0	1	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando noctiflora	3	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	0	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando owariensis	3	1	0	0	0	1	-	0	0	0	2	0	0	1	0	0	0	0	0	1	1	0	2	0	1	1	0	1	2	1	1	0	1	0	1	0	1	0	0	0
Lando parviflora	1&3	1	0	1	0	1	0&1	0&1	0	0	1	3	0	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Lando reticulata	3	1	0	1	0	0	1	1	0	0	2	3	0	1	0	0	0	0	0	1	1	0	2	0	1	1	0	0	2	?	1	0	1	0	1	0	1	0	0	0
Lando subrepanda	3	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	1	1	0	0	0	0	0	1	0	0	0
Lando tenuis	3	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	0	0	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0

Appendix 4. Continued

	Character states																																						
	0									1										2										3									4
Taxon	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0
Lando tholonii	1&3	1	0	1	1	1	0&1	0&1	0	0	0	0	0	1	0	0	1	0	0	1	1	1	2	0	1	0	0	1	2	1	1	0	1	0	1	0	1	0	0	0
Leuco anceps	3	1	0	1	0&1	0	-	0	0	2	2	1	0	0&1	0	1	0&1	0	0	0	1	0	2	0	0	0	0	0	2	1	1	0	0	0	0	0	0	1	0
Leuco eugenii613	3	1	0	1	0&1	1	-	0	0	2	2	2	0	1	0	1	0&1	0	0	0	1	1	2	0	0	0	0	0	2	1	1	0	0	0	0	0	0	1	0
Ortho barteri	3	1	0	1	1	1	0	1	0	0	0	0&1	1	1	1	0	0	0	0	0	1	0	2	1	0	1	0	1	2	0	1	0	2	0	1	0	1	0	0	0
Ortho chirrosa	3	1	0	0	1	0&1	0	1	0	0	0	0&1	1	1	1	0	0	0	0	0	1	0	2	1	0	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Ortho indeniensi	3	1	0	0	1	0&1	0	1	0	0	0	0&1	1	1	1	0	0	0	0	0	1	0	2	1	1	1	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Ortho visciflua	3	1	0	1	1	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	0	2	1	0&1	1	0	1	2	0	1	?	?	0	1	0	1	0	0	0
Pacou bolivie373	3	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Pacou bolivie463	3	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Pacou bolivie603	3	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Pacou bolivie588	3	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Parah fasciculat	0	1	0	0	0	0	-	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	2	0	1	?	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Parah peruviana	0	1	0	0	0	0	-	0	0	0	0	0	0	?	0	0	0	0	0	?	1	?	2	0	1	?	0	1	2	0	1	0	0	0	0	0	1	0	0	0
Parah negroensis	0	1	0	0	0	0	-	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	2	0	1	?	0	1	2	0	1	0	1	0	0	0	1	0	0	0
Parah sp531	0	1	0	0	0	0	-	0	0	0	0	?	0	?	0	0	0	0	0	?	1	?	2	0	1	?	?	1	2	0	1	0	?	0	0	0	1	0	0	0
Parah surrogata	0	1	0	0	0	0	-	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	0	1	2	0	1	2	0	1	0	1	0	0	0	1	0	0	0
Saba comorensis	3	1	0	0	0	0	0	1	0	0	0	0	1	0&1	0	2	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	1	0	0
Saba florida	3	1	0	0	0	0	0	1	0	0	0	0	1	0&1	0	2	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	1	0	0
Saba senegalens	3	1	0	0	0	0	0	1	0	0	0	0	1	0	0	2	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	1	0	0
Saba thompsonii	3	1	0	0	0	0	0	1	0	0	0	0	1	0	0	2	0	0	0	0	1	0	2	0	1	1	0	1	2	0	1	0	1	0	1	0	1	1	0	0
Vahad caillei	3	1	1	0	0	0	1	1	0	2	0	0	1	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Vahad laurentii	3	1	1	0	0	0	1	1	0	2	0	0	0	1	0	0	0	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	0	0
Willu angustifol	3	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	1	0	?	0	?	2	0	1	0	1	0	0	0	0	0	0	0
Willu anomala	3	1	0	0	0&1	0	0	1	0	0	0	1	1	0&1	0	0	0	0	0	1	1	0	2	1	0	?	0	?	2	0	1	0	1	0	0	0	0	0	0	0
Willu beccariana	3	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	2	1	0	?	0	1	2	0	1	0	1	0	0	0	0	0	0	0
Willu coriacea	3	1	0	0	1	0	0	1	0	0	0&2	0	0	0&1	0	0	0	0	0	1	1	0	2	1	0	0	0	1	2	0	1	0	1	0	0	0	0	0	0	0
Willu edulis	3	1	0	0	1	1	0	1	0	0	0	2	1	0&1	0	0	0	0	0	1	1	0	2	1	0	?	0	1	2	0	1	0	1	0	0	0	0	0	0	0
Willu flavescens	3	1	0	0	1	0	0	1	0	0	2	0&1	0	0	0	1	0	0	0	1	1	0	2	1	0	?	0	?	2	0	1	0	1	0	0	0	0	0	0	0
Willu sarawacens	3	1	0	0	1	1	0	1	0	0	0	2	1	1	0	0	0	0	0	1	1	0	2	1	0	?	0	?	2	0	1	0	1	0	0	0	0	0	0	0

CAPÍTULO 2

HISTORICAL BIOGEOGRAPHY OF THE VIWITA PANTROPICAL CLADE (APOCYNACEAE, RAUVOLFIOIDEAE)

ABSTRACT

Aim The ViWiTa clade belongs to the plant family Apocynaceae and comprises representatives of the three largest tribes (Vinceae, Willughbeieae, Tabernaemontaneae) within subfamily Rauvolfioideae. The clade is distributed mainly in tropical regions of the world, with only one genus in temperate zones. The main goal of this study is to present a molecular dating and biogeographical analyses of this group to assess its temporal and spatial origins, testing possible vicariant patterns and dispersal routes.

Location Pantropics.

Methods Molecular age estimates were calculated using a Bayesian approach based on 100% generic sampling of the ViWiTa clade and five chloroplast markers. Ancestral areas were reconstructed using a maximum likelihood approach that implements the dispersal-extinction-cladogenesis model.

Results Dating analyses indicate that the ViWiTa clade probably originated in the Early Paleocene in Australasia, with subsequent colonizations events to the Neotropics, Africa and Madagascar. A single dispersal event from Africa to the Neotropics and from the Neotropics to Hawaii during the Miocene was also inferred.

Main conclusions The estimated crown node ages of the ViWiTa clade in combination with ancestral area analysis imply a scenario congruent with an Australasian origin followed by migration via the Boreotropics from Early to Middle Eocene, and further dispersal events into South America. Others dispersal events (either across the sea or facilitated by postulated land bridges) are required to explain disjunctions between Madagascar and Australasia in *Tabernaemontana* and between Australasia and Africa in *Tabernaemontana* and Willughbeieae. Multiple long-distance dispersal events were inferred.

Keywords Intercontinental disjuctions, long-distance dispersal, Boreotropics, Neotropical, Palaeotropical.

INTRODUCTION

The Vinceae-Willughbeieae-Tabernaemontaneae clade (ViWiTa) comprises representatives from the largest tribes of Rauvolfioideae, Apocynaceae, with 42 genera and 470 species distributed in tropical regions, with exception of *Vinca,* distributed in temperate regions. Species from this ViWita clade exhibit a remarkable morphological variation, ranging from herbs to shrubs and lianas, medium understorey trees to giant canopy trees. Fruits are dehiscent or indehiscent, fleshy or dry; and seeds naked, winged, arillate or comose.

Previous studies support the monophyly of the ViWiTA clade and its tribes (Simões *et al.*, 2007, 2010; Chapter 1). Species of ViWiTa are distributed in the Neotropics with 168 species, Afrotropics with 156 and Australasia with 146. However, the diversity of species is different within each tribe. Vinceae is most diverse in Australasia, whereas Willughbeieae has the largest number of species in tropical Africa and Tabernaemontaneae in the Neotropics.

The broad phylogenetic study of Vinceae carried out by Simões *et al*. (in prep.) recognizes six natural groups that correspond to six subtribes. The first subtribe to diverge is Kopsiinae, comprising a single genus, *Kopsia*, found from southern China and Burna to northen Australia and Nanuatu and is most diverse in Peninsular Malaysia and Borneo. All species of *Kopsia* are shrubs or small trees at forest edges or in the open, and fruits are small drupes often with a lateral spur (Middleton, 2004). Ochrosiinae, with one genus, *Ochrosia,* is widespread in tropical Asia and Oceania with some species occurring in the Marquesas and Hawaiian Islands and two distributed in Africa, Mascarene Islands and Seychelles

(Middleton, 2007). *Tonduzia* and *Vinca* are from Neotropical and temperate regions respectively, with follicular fruits. Despite the monophyly of these genera, their phylogenetic position within Vinceae is uncertain (Simões *et al*. unpubl. data). Catharanthinae is a tribe with an interesting disjunction pattern between *Kamettia* from Western Ghats of India and Thailand (Middleton, 2005) and Malagasy *Catharanthus* and *Petchia*. Petchia has six endemic Malagasy species, one endemic to Sri Lanka and one in continental Africa (Leeuwenberg, 1997). Subtribe Rauvolfiinae comprises a single genus (*Rauvolfia*), with a broad pantropical disjunction, with some species reaching Hawaii.

According to Simões *et al.* (2010), Tabernaemontaneae is divided into two subtribes, Ambelaniinae and Tabernamontaninae. Ambelaniinae is the first lineage to diverge and comprises six Neotropical genera restricted to lowland tropical Amazonian forest. Species of Ambelaniinae are small to medium sized trees with indehiscent fruit, some of wich are appreciated by primates (Monachino, 1945; Schultes, 1979; Roosmalen, 1985), and seeds without aril. Tabernaemontaninae, on the other hand, is Pantropical and can be divided in two subclades, named Callichilia and Tabernaemontana clades. The Callichilia clade has seven genera restricted to Africa with some species of *Voacanga* reaching Asia, and species have baccate or follicular fruits. The Tabernaemontana clade has only one genus, *Tabernaemontana*, with intercontinental disjuction in which, sub-clades within it correspond to four large geographic areas: Neotropics, Madagascar, Africa and Asia. Species of *Tabernaemontana* are shrubs or small trees, ocurring mainly in wet tropical flora, with fleshy fruits and seeds often surrounded by a colorful aril, which are

dispersed mainly by birds and primates (McDiarmid, 1977; Roosmalen, 1985; Baraloto & Forget, 2007).

The phylogenetic study of Willughbeieae presented in Chapter 1 had as result four clades that correspond to the four subtribes recognized by Endress *et al.* (2014): Leuconotidinae, Willughbeiinae, Lacmelleinae and Lanolphiinae. Leuconotidiinae is a small subtribe with three genera and seven species, all of them lianas with baccate fruits. *Bousigonia* and *Leuconotis* are distributed in tropical Asia (Leeuwenberg, 2002), while *Cyclocotyla* is restricted to West and Central tropical Africa, in riverine forests (Van de Ploeg, 1985). Species of Lacmelleinae, on the other hand, are shrubs or trees from the Neotropics, mainly occuring in rain forests, with baccate fruits, most of them edible. Willughbeiinae is monogeneric, with all species of *Willughbeia* restricted to Southeasten Asia. They are lianas with tendrils formed from modified inflorescences, fleshy baccate fruits, some of them edible (Middleton, 1993), and is sister group to African Landolphiine. Most species of Landolphiinae are lianas, more rarely shrubs, commonly found near to rivers, and have edible baccate fruits. Landolphiinae show a disjunct distribution, with most species (ca. 60 spp.) distributed in Africa and Madagascar and three species in the Neotropics.

The ViWita clade is an ideal group for biogeographic studies, due to the distribution of its species in all major tropical regions of globe and a considerable variation in morphological traits. Also, there is a phylogenetic hypothesis to each tribe based on a broad taxon sampling with representatives of all genera and the majority of the valid species (Simões *et al*., 2010, Simões *et al.*, unpubl. data, Morokawa *et al*., Chapter1).

The goal of this study is to reconstruct a biogeographic history of the ViWiTa clade. Our initial hypothesis is that similar disjunction patterns in different tribes took place simultaneously, driven by similar colonization routes.

MATERIALS AND METHODS

Data sets and sampling:

We analysed a dataset comprising 288 specimens from 42 genera recognized in ViWiTa clade. All genera recognized in ViWiTa were sampled, and the percentage of species sampled in each genus was around 40-60%. These sequences were obtained from previous works in Tabernaemontaneae (Simões *et al*., 2010), Vinceae (Simões *et al*., unpubl. data) and Willughbeieae (Morokawa *et al*., unpubl. data, Chapter 1). For this study we added sequences from 13 species of tribe Tabernaemontaneae to improve the taxonomic sampling. Voucher informations is listed in Appendix 1. Four species from the three genera belonging to tribes Aspidospermeae (*Aspidosperma)* and Alstonieae (*Alstonia* and *Dyera*) were selected as outgroups based on previous studies (Simões *et al*., 2007; Simões *et al*., 2010).

The dataset included five chloroplast regions: *rpl16*, *rps16* and *trn*K introns; *trn*S-G intergenic spacer and *mat*K gene. All data were combined into one data set and sequence alignment was performed using MAFFT version 7 (Katoh & Standley, 2013) with defaults parameters and the strategy used was L-INS-i. The final matrix had 7307 aligned characters.

Molecular dating

The suitability of the clock-likeness of the data was assessed using Bayes factor comparisons between a strict molecular clock and a lognormal relaxed clock. Bayes factor is the ratio of the marginal likelihoods of two models, used to model comparison and model selection in a Bayesian framework (Drummond & Rambaut, 2007). The software Tracer version 1.5 (Rambaut & Drummond, 2009) was used to calculate Bayes factors. The evidence against a strict molecular clock was strongly rejected against a lognormal relaxed clock using the categories proposed by Kass & Raftery (1995). We thus estimated node ages of the ViWiTa clade using a Bayesian relaxed clock model as implemented in BEAST 1.7.4 (Drummond & Rambaut, 2007). The selected model of nucleotide substitution following the Bayesian information criterion (BIC) was GTR + gamma by using jModeltest 2.1.1 (Darriba *et al.*, 2012). A Birth-Death Process speciation tree prior was specified. Four independent Markov chain Monte Carlo (MCMC) runs of 50 million generations with trees sampled every 5000 generations were performed on CIPRES Science Gateway portal v3.3 (http://www.phylo.org/index.php/portal/). Tracer version 1.5 (Rambaut & Drummond, 2009) was used to check for convergence between the runs and to determine the burn-in phase. Results were considered reliable once the effective sampling size (ESS) for all parameters exceeded 200. The sampled posterior trees were summarized onto a maximum clade credibility tree using the program TreeAnnotator 1.7.4 (Drummond *et al.*, 2012).

For an accurate estimation of divergence times it is necessary multiple fossil calibrations, accuracy in phylogenetic reconstruction using many genes and taxa as possible, and missing data can influence on phylogenetic inference

and branch length estimation (Sauquet, 2013). There are four main kinds of sources of calibration, but the most usual is the fossil record and the major challenge is to know where place the fossil within a phylogeny. Other option is use a secondary calibration, where dates obtained in a previous molecular dating study is used to calibrate the target group. This approach is generally used in absence of fossils. However this kind of source can increase error and uncertainty in estimated ages (Graur & Martin, 2004).

Within ViWiTa clade just one reliable fossil was found, and to have an accurate estimate is necessary multiples fossil. If we used others fossil in Apocynaceae, we need to sample other genera related with the point of calibration in the same proportion, other way is find in GenBank the sequences with the same regions used in the study, probably the number of sampled species per genera would be reduced, due the absence of sequences.

The age of ViWiTa root (62.2 Ma, 95% HPD 53.4-69.9 Ma) was originated of a large study of Gentianales ordem (Scharn *et al*. in prep), this study used a supermatrix with 430 tips, three genes and 38 fossils with new approach to include fossil in biogeographic analyses.

We assigned the root node a normally distributed prior with a mean of 1 and standard deviation of 0.1. After that, we rescaled the root node using the absolute age of ViWiTa node using the R packages GEIGER (Harmon *et al.*, 2008) and Diversitree (FitzJohn, 2012).

Biogeographical analysis

Ancestral area reconstruction

The operational areas were based on the extant distribution patterns and in areas adapted from Holt *et al*. (2013). We defined six areas: Neotropical (South America, and tropical North America including West Indies), Afrotropical (sub-Saharan Africa), Australasia (north Australia, New Caledonia, New Zealand, tropical and subtropical Asia, and Oceania), Madagascan (Madagascar, Seychelles islands, Mascarene and Comoro islands), Paleartic (boreal and temperate Eurasia as well as northern Africa), and Hawaii (Fig. 1).

To estimate ancestral areas, we used the maximum likelihood dispersal–extinction–cladogenesis (DEC) method (Ree et al., 2005; Ree & Smith, 2008) as implemented in LAGRANGE build 20130526 (Ree & Smith, 2008), based on the maximum clade credibility chronogram. The likelihood of all possible biogeographical scenarios was calculated at each internal node of the phylogeny. Biogeographical reconstructions lying within a confidence interval of two log-likelihood units were considered most reliable (Ree & Smith, 2008). The maximum ancestral area size was constrained to 2 because there are no species in our analyses distributed in more than two areas.

RESULTS

The run number two of the BEAST analysis was chosen among four independent runs, because it showed highest likelihood (Fig. 2), ESS parameters values exceeding 200 and all parameters converged on a stationary distribution.

The BEAST analysis generated a well-supported tree for ViWiTa (Fig. 3, Appendix 2), which was consistent with topologies from the previous studies. The

chronogram with the median age and 95% HPD (Highest Posterior Density) of the selected clades is summarized in Fig. 3 and Table 1.

The molecular clock estimation for the crown age of the Vinceae (51.6 Ma; 95% HPD 44.2-57.9; node 3 in Fig. 3) and Willughbeieae clades (50.6 Ma; 95% HPD 41.8-56.3; node 4 in Fig. 3) lies within the boundary of the Early Eocene. The initial diversification of Tabernaemontaneae crown clade was estimated to occur in the Late Eocene (34.1 Ma; 95% HPD 26.9-42.6; node 5 in Fig. 3).

Ancestral area analyses

Results from the DEC analyses are summarized in Table1. Alternative biogeographical scenarios are shown when relative probability was less than 0.80.

The most likely ancestral area for ViWiTa was Australasia (node 1 in Fig1; Table 1). The ancestral area of the most recent common ancestor (MRCA) of Vinceae and Willughbeieae was likewise inferred as Australasia, whereas the ancestral area of Tabernaemontaneae was inferred as both Neotropical and Australasia. The Neotropical colonization occurred at least four times from Australasia (nodes 5, 6, 7, 10 in Fig. 3; Table 1) and once from the Afrotropics (node 16 in Fig. 3; Table 1). We found at least four events of colonization from Australasia to the Afrotropics (nodes 9, 12, 15, 20 in Fig. 3; Table 1) and one Afrotropcis back to Australasia (node 23 in Fig. 3; Table 1). The Madagascan colonization occurred twice from Australasia (nodes 8, 18 in Fig. 3; Table 1), and twice from the Afrotropics (node 21, 27 in Fig. 3; Table 1) and a recolonisation to Australasia from Madagascar (node 24 in Fig. 3; Table 1). The Hawaiian

colonization occurred twice from the Australasia (nodes 22, 25 in Fig. 3; Table 1) and once from Neotropics (node 17 in Fig. 3; Table 1).

The 13 species of *Tabernaemontana* included in the phylogeny were nested within the *Tabernaemontaneae* clade and therefore did not change the generic relationship within the tribe, when compared with the phylogeny of Simões *et al*. (2010).

DISCUSSION

Australasia → Neotropics

Disjunct distributions between Neotropics, Africa and Asia of clades younger than Gondwanan break-up (180-100 Ma; McLoughlin, 2001) are currently often assumed to be the result of a migration through the Boreotropical flora (Wolfe, 1975). Studies with molecular dating analyses, based on a number of taxa, found that splitting time of continental disjunctions in tropical clades are consistent with the disruption of Boreotropical ranges around the Eocene-Oligocene boundary, for example in Malpighiaceae (Davis *et al*., 2002), Meliaceae (Muellner *et al*, 2006), Melastomataceae s. str. (Renner *et al*, 2001), Rubiaceae (Antonelli *et al.*, 2009), Urophylleae (Rubiaceae, Smedmark *et al*. 2010), Annonaceae (Couvreur *et al*., 2011), and *Cissus* (Vitaceae, Liu *et al.*, 2013). Such scenario may explain three disjunctions found in ViWiTa, as in the first split in the Tabernaemontaneae clade at c. 34.1 Ma (node 5 in Fig. 3, Table 1), in Vinceae clade at c. 33.1 Ma (node 6 in Fig. 3, Table 1), in Willughbeieae clade at c. 30.7 Ma (node 7 in Fig 4, Table 1).

We found two splits between the Neotropics and Asia in *Rauvolfia* at c. 26.2 Ma (node 6 in Fig. 3, Table 1) in the Late Oligocene and in *Tabernaemontana* at c. 21.7 Ma (node 14 in Fig. 3, Table 1) in the Early Miocene, wich, that could be explained by direct transoceanic dispersal across the Pacific.

Australasia ↔ Africa

Four different scenarios are commonly hypothesized to explain African–Asian disjunctions: (1) Eocene 'rafting' of biota on the Indian tectonic plate, (2) Eocene boreotropical forests in southern Europe, (3) Dispersal across Arabia and central Asia via the tropical forest that developed during the late Middle Miocene thermal maximum (17-15 Ma), and (4) Transoceanic long-distance dispersal (LDD).

The first and second scenarios are not congruent with the comparatively late dispersal age inferred here. The third scenario could explain three events of colonization from Australasia to Africa in Miocene. The collision of Africa and Arabia with Eurasia during the Miocene led the formation of a migration route, termed the Gomphotherium land bridge, wich supposedly allowed the migration of flora and fauna between Africa and Eurasia (Rögl, 1999). This route would have enabled the migration of the MRCA of the *Willughbeia+Landolphia* at c. 25.8 Ma (95% HPD 19.8-31.0 Ma, node 12), MRCA of *Leuconotis+Cyclocotyla* at c. 19.9 Ma (95% HPD 11.3-34.9 Ma, node 15) and in Asian *Tabernaemontana* at c. 12.8 Ma (95% HPD 9.3-17.2 Ma, node 20 in Fig. 3). This route was used to explain the dispersal from Africa to Asia in *Uvaria* (Zhou *et al*., 2012).

The colonization from Africa to Australasia was observed just once in species of *Voacanga* in the Tabernaemontaneae clade, at c. 6.1 Ma (95% HPD 3.2-10.5 Ma, node 23 in Fig. 3), and could be explained by transoceanic LDD, due to very recent origin. In the literature, many African-Asian disjunctions, have been explained by LDD, in both directions: Asia to Africa in *Bridellia* (Phyllantaceae) at 10 Ma (Li et al. 2009); *Macaranga* and *Mallotus* (Euphorbiaceae) < 27 Ma (Kulju et al. 2007); *Paederia* (Sapotaceae) 15.9 (Nie et al. 2013); Africa to Asia in *Osbeckia* (Melastomataceae) at c. 7-16 Ma (Renner & Meyer, 2001a; Renner, 2004); *Gaertnera* (Rubiaceae) at c. 5-6 Ma (Malcomber, 2002); *Exacum* (Gentianaceae) less than c. 35 Ma (Yuan et al., 2005); *Cucumis* (Curcubitaceae) less than c. 10 Ma (Renner et al., 2007); *Uvaria* (Annonaceae) at 16.1-21.4 Ma (Zhou et al., 2012); *Cissus* (Vitaceae) 7.8-25.6 Ma (Liu et al., 2013). The migration from Africa to Asia is more frequent than Asia to Africa. The LDD mechanism in *Voacanga* could be by oceanic currents, due to its widespread distribution in the islands of Malesia (12 spp.), wich probably originated from Africa, where seven species occur.

Australasia ↔ Madagascar ← Africa

This pattern of disjunction between Australasia, Madagascar and Africa could be explained by the break-up of Gondwana c. 165 Ma (Magallón *et al*., 1999; Crane *et al*., 2005), or 'rafting' on the Indian tectonic plate 94-65 Ma (Conti *et al*. 2002), migration through the Eocene Boreotropical Flora (Wolfe, 1975), or Eocene-Oligocene dispersal from India to Madagascar through the 'Lemurian stepping stones' across the Indian Ocean (Schatz, 1996).

The MRCA of Malagasy *Tabernaemontana* species (Tabernaemontaneae clade in Fig. 3) is inferred to Australasia and the split was c. 14.3 Ma (95% HPD 10.4-19.2, node 18 in Fig. 3) in the Early Miocene, this age is too young to have been influenced by break-up of Gondwana, Indian rafting or dispersal through the Eocene Boreotropical Flora. LDD across the western Indian Ocean seems to be the most likely explanation for the disjunction of *Tabernaemontana* from Australasia to Madagascar. Others studies also suggested LDD to this pattern, as in *Paederia* (Nie *et al*. 2013) and *Bridelia* (Li *et al.*, 2009). Asian species of *Tabernaemontana* have a broad distribution in tropical Asia, both continental and insular, from southwestern India until northern of Australia (Leeuwenberg, 1991). This wide distribution could suggest a capacity to dispersal through to islands, and the seeds with aril may have facilitated LDD by birds.

In the Vinceae clade the colonization from Australasia to Madagascar could have been through dispersal via 'Lemurian stepping stones' in the clade formed by *Kamettia*, *Petchia* and *Catharanthus*. The split between the *Kamettia* and the others two genera was in the late Oligocene 27.9 Ma (95% HPD 21.3-36.2, node 8 in Fig. 3). *Kamettia* is a small genus with two species, *K. caryophyllata* (Rooxb.) Nicolson & Suresh, which is found in the Western Ghats of India and *K. chandeei* in south-western Thailand, both are liana, with follicular fruits and flattened winged seeds (Middleton *et al*., 2005).

Petchia has eight species, six species endemic to Madagascar, one endemic to Cameroun and one endemic to Sri Lanka, *P. ceylanica* (Wight) Livera. The colonization of *P. ceylanica* to Sri Lanka from Madagascar dates of 3.2 Ma (95% HPD 1.0-6.7 Ma, node 24 in Fig. 3), AND this recent colonization

could be explain by LDD through birds, since the fruits in this species are reddish formed by two mericarps articulated into single-seeded drupaceous segments, and therefore adapted to bird dispersal. *Catharanthus* has a distribution similar to *Petchia* with seven species endemic to Madagascar and one restricted to India and Sri Lanka, however, we have not sampled the *Catharanthus* species from India.

Within *Rauvolfia* another dispersal event is observed, a split between Malagasy and African species dated of middle Miocene, 12.5 Ma (95% HPD 9.0-18.2 Ma, node 21 in Fig. 3). Magalasy *Rauvolfia* has probably as ancestral area Africa, however, because of the low support of branchs it is not possible to infer precisely.

A general lack of support for the phylogenetic relationships among the Malagasy species of *Landolphia* (Willughbeieae clade, node 26 in Fig. 3) makes the time of colonization unclear. The MRCA of these species is probably from Africa and the age is less than crown age of *Landolphia* (95% HPD 9.7-17.5 Ma, node 27 in Fig. 3). However, our results do not allow us to make reliable predictions about the number of colonization events between Africa and Madagascar within *Landolphia* .

Australasia → Hawaii ← Neotropics

The Hawaiian archipelago on the Pacific Plate is 3500 km distant from the nearest landmass. Such isolation suggest that LDD plays an important role on the origin of the hawaiian flora (Keeley & Funk, 2011). The majority of the Hawaiian flora originated from south and west Pacific lineages (Howarth *et al*., 1997; Wright

et al., 2001, Cantley *et al*., 2014). In the ViWiTa clade, the colonization of Hawaii is restricted to the Vinceae clade, since no representatives of both Tabernaemontaneae and Willughbeieae clades are found on the archipelago. Within *Rauvolfia*, the dispersal event into Hawaii dates from the middle Miocene, 15.3 Ma (95% HPD 8.5-20.8 Ma in node 17 in Fig.4), probably from the Neotropics. The Hawaiian *Rauvolfia* species are sister to a group of Cuban species. We hypothesise that the ancestral of this clade was in Central America in the Miocene, when the sea currents could have facilitated the transport to Hawaii before the closure of Isthmus of Panama. An example of LDD by seed or capsule flotation in oceanic currents from Mesoamerica to Hawaii is *Gossypium tomuntosum*, Malvaceae (DeJoode & Wendel, 1992).

In *Ochrosia* there were two events of colonization from Australasia in the late Miocene, 6.4 Ma (95% HPD 3.4-11.6 Ma, node 22 in Fig.4) and in Pliocene, 3.1 Ma (95% HPD 1.3-6.3 Ma, node 25 in Fig. 3). *Ochrosia* species are widespread in mostly coastal areas and lowland forest in the Pacific Islands (Hendrian, 2004). This widespread distribution can be associated to the capacity of fruits to dispersal by water, since the fruits of *Ochrosia* have a fribrous wall, a dense network of hard sharp fibres diverging from the endocarp suggesting that water is the major dispersal agent for fruits of this genus (Simões *et al.* in prep.).

Afrotropics → Neotropics

Landolphia is the most diversified clade of Willughbeieae, with about 60 species. Our phylogenetic study (chapter 1) showed that *Landolphia* and a number of morphologically alike genera distributed in tropical Africa form a

strongly supported clade together with species from the neotropical genus *Pacouria*. The crown age of Neotropical species of *Landolphia* is 17.0 Mya (95% HPD 12.6-19.7, node 16 in Fig. 3) in the early Miocene. This divergence time is well after the last possible connection of Africa and South America, around 96-105 Mya (McLoughlin, 2001). We thus suggest that LDD is the most plausible mechanism for this trans-Atlantic disjuction. Most of tropical trans-atlantic dispersal events probably took place by surface sea currents (Renner, 2004). However, the fruits of *Landolphia* are baccate and this kind of fruit is not adequate to dispersal through water. An interesting phenomenon observed is the floating islands from tropical Atlantic deltas of Congo, Senegal, and Amazon rivers, across the Atlantic Ocean (Renner, 2004). These islands can transport plants or part of plants. Most species of *Landolphia* occurr in riverine vegetation, and its fruits might have been lead out of Africa through floating islands.

In other works we can find the same pattern of long distance dispersal from Africa to South America via water, such as in *Symphonia globulifera* (Clusiaceae) around 17 Ma (Dick *et al*., 2003); and *Renealmia* (Zingiberaceae) 15.8-2.7 Mya (Sarkinen *et a.l*, 2007).

CONCLUSIONS

The three tribes that comprise the ViWiTa clade have similar disjunct distribution patterns. We were interested whether disjunction in these tribes happened at the same time, possibly driven by the same process. The first disjunction in each tribe was between Australasia and Neotropics, these splits happened almost in the same period and can be explained through of rupture

of Boreotropical flora existent in Eocene. Others disjunction patterns repeat in each tribe, but sometimes not in the same time and direction, and some patterns are exclusive of one tribe, for example, the colonization of Hawaii in Vinceae tribe, colonization from Africa to the Neotropics in Willughbeieae.

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Figure 1. Map of regions coded for taxa in ancestral area analysis. **A** = Afrotropical, yellow; **H** = Hawaii, black circle; **M** = Madagascan, red; **N** = Neotropical, green; **P** = Palearctic, gray; **U** = Australasia, orange.

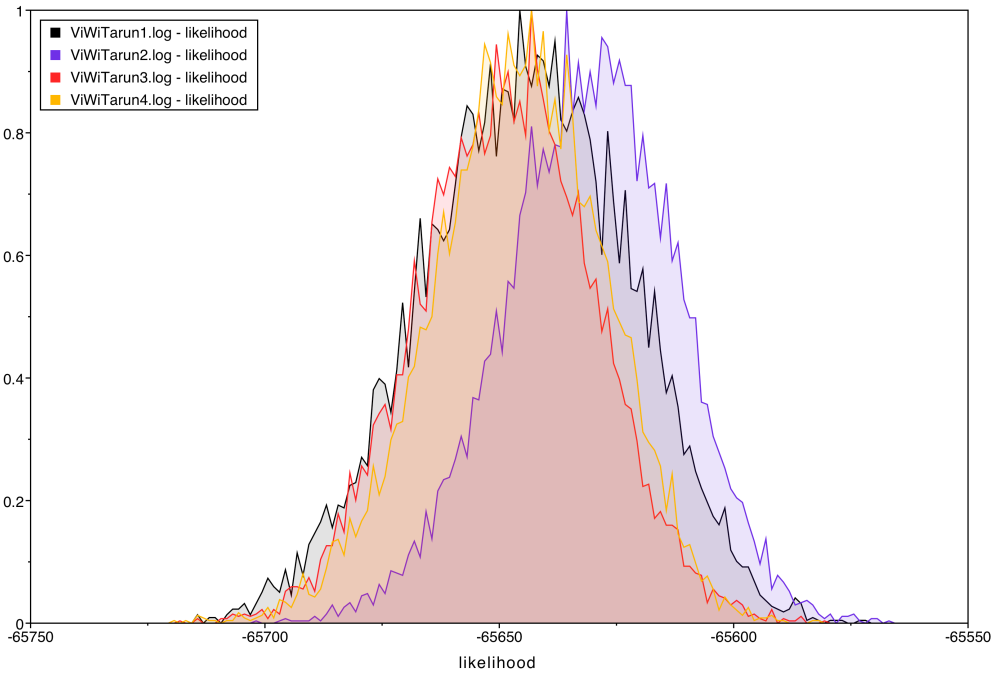


Figure 2. Marginal density of likelihood parameter to four runs on Beast.

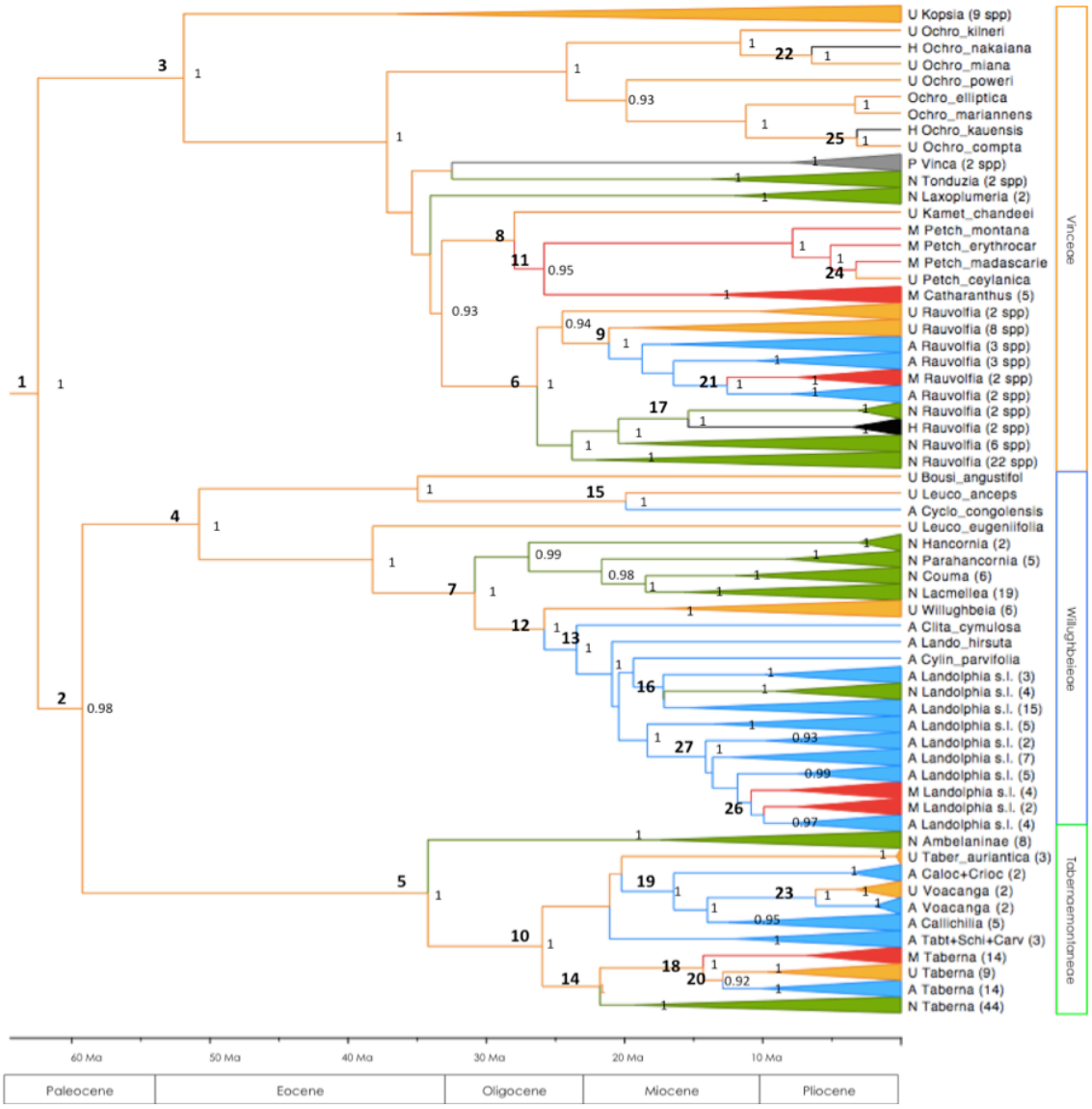


Figure 3. Chronogram of ViWiTa clade based on plastid sequence (rps16, rpl16, trnS-G, trnK and matK) as inferred from BEAST obtained under a relaxed clock. The clades with the same disribution were collapsed to reduce the size of tree for visualization (see fully annotaded chronogram in Appendix 2). The black numbers at nodes are Bayesian posterior probabilities ≥ 0.9. The black numbers in bold at branch refers to those in Table 1. The colors correspond to the areas in the map in Fig.1. The branches were colored based on ancestral area

reconstruction results. A = Afrotropical, blue; H = Hawaii, black; M = Madagascan, red; N = Neotropical, green; P = Palearctic, gray; U = Australasia, orange.

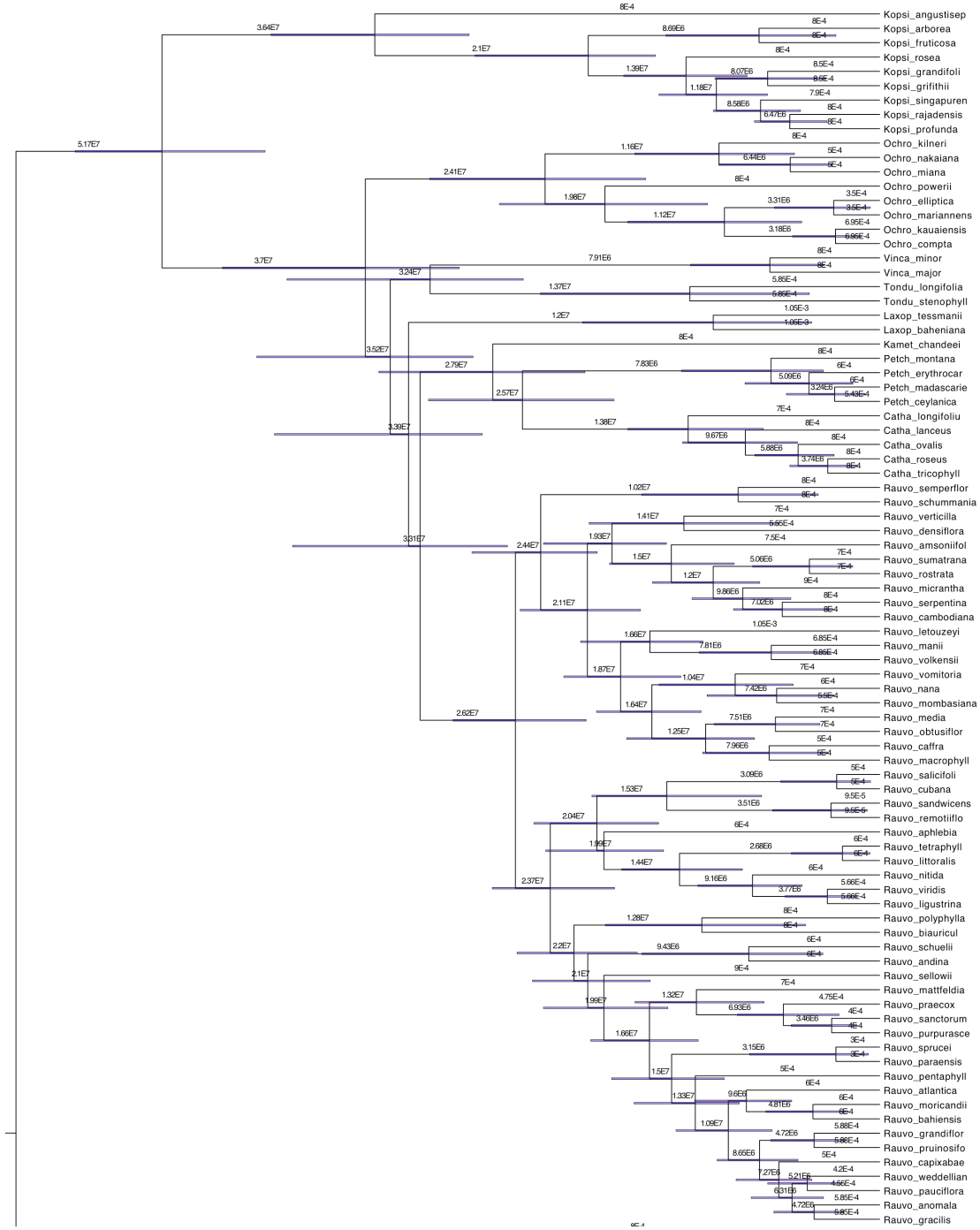
Table 1. Estimated ages and ancestral area reconstructions of selected clades in ViWiTa, with 95% credibility intervals (Highest Posterior Density, HPD). Node numbers refer to those in Fig. 3, left panel. Alternative biogeographical scenarios are shown when relative probabilities were smaller than 0.80 in Lagrange (dispersal-extiction-cladogenesis, DEC), right panel. A = Afrotropical; H = Hawaii; M = Madagascan; N = Neotropical; U = Australasia.

	Age estimates		
Node	Median (Ma)	95% HPD(Ma)	Lagrange (Rel. Prob.)
1: Viwita crown	62.2	53.4-69.9	U|U (0.88)
2: Wita crown	59.0	54.8-62.0	U|U (0.84)
3: Vinceae clade	51.6	44.2-57.9	U|U (0.87)
4: Willughbeieae clade	50.6	41.8-56.3	U|U (0.87)
5: Tabernaemontaneae clade	34.1	26.9-42.6	NU|U (0.87)
6: *Rauvolfia* crown	26.2	21.1-30.7	N|U (0.99)
7: Lacmelleinae clade	30.7	24.9-37.4	N|U (0.82)
8: *Kamettia*	27.9	21.3-36.2	U|U (0.66) U|M (0.24)
9: *Rauvolfia* subclade	21.1	17.3-25.9	U|A (0.99)
10: Tabernamontaninae	25.8	21.1-31.9	NU|U (0.88)
11: *Petchia+Catharanthus*	25.8	19.2-32.6	UM|M (0.89)
12: *Willughbeia+Landolphia*	25.8	19.8-31.0	U|A (0.82)
13: *Landolphia* s.l. crown age	23.5	18.7-28.4	A|A (0.82)
14: *Tabernaemontana*	21.7	17.2-25.7	U|N (0.91)
15: *Leuconotis+Cyclocotyla*	19.9	11.3-34.9	U|A (1.00)
16: *Pacouria*	17.0	12.6-19.7	N|A (1.00)
17: N60 Hawaii *Rauvolfia*	15.3	8.5-20.8	N|H (1.00)
18: Paleotropical *Tabernaemontana*	14.3	10.4-19.2	M|U (0.92)
19: N542 *Vocanga+Callichilia*	14.0	9.0-19.3	AU|A (0.76) A|A (0.19)
20: Asian *Tabernaemontana*	12.8	9.3-17.2	A|U (1.00)
21: Magalasy *Rauvolfia*	12.5	9.0-18.2	M|A (1.00)
22: *Ochrosia nakaiana*	6.4	3.4-11.6	H|U (1.00)
23: Asian *Voacanga*	6.1	3.2-10.5	A|U (1.00)
24: *Petchia*	3.2	1.0-6.7	M|U (1.00)
25: *Ochrosia kauensis*	3.1	1.3-6.3	H|U (1.00)

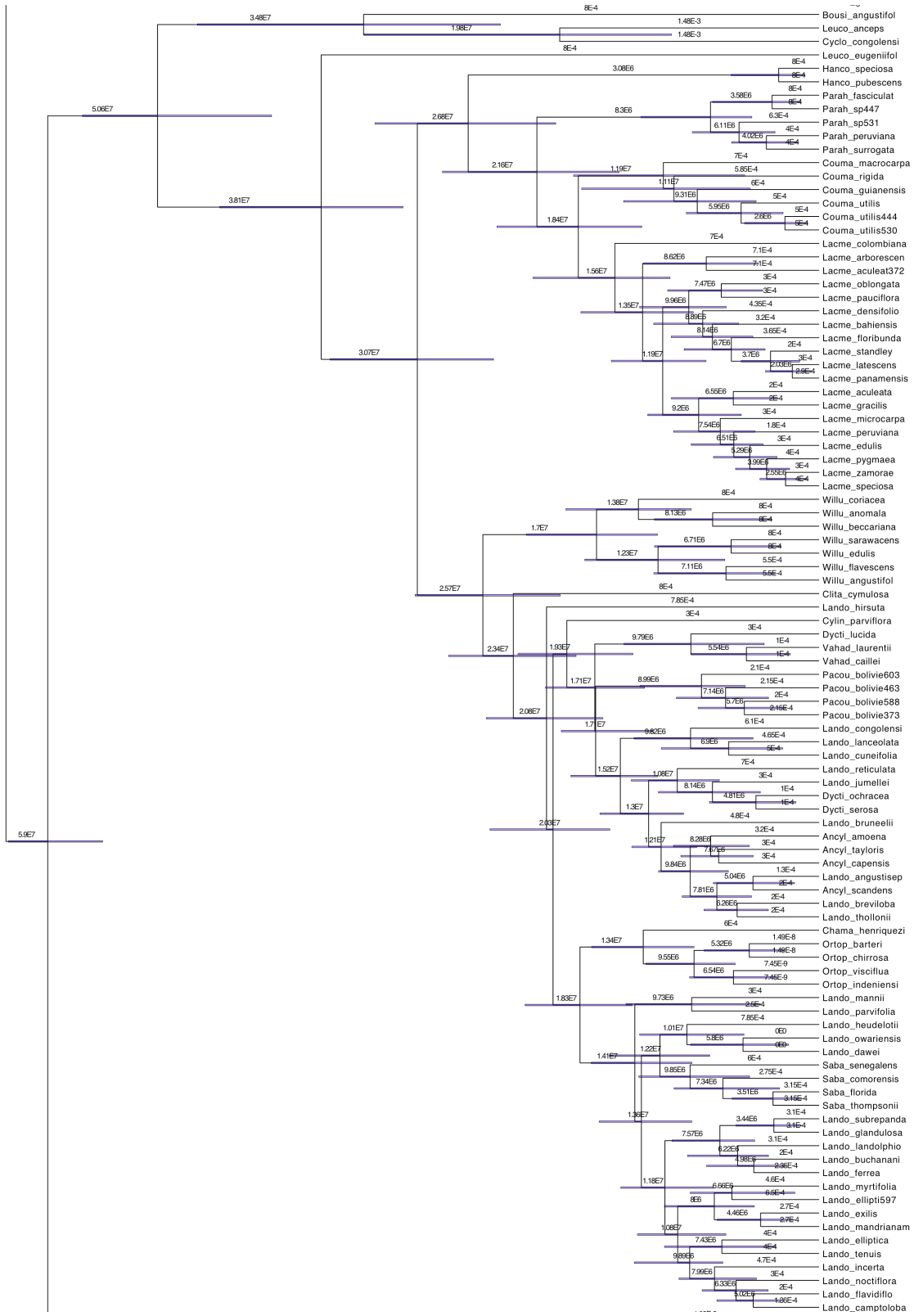
Appendix 1: Voucher information of species added.

Species	**Locality**	**Collector (Herbarium)**
Ambelania occidentalis Zarucchi	Peru, Polcazu.	*R. Vásquez et al. 30702* (INB, MO)
Tabernaemontana pauli (Leeuwenb.) A.O. Simões & M.E. Endress	Costa Rica	
Tabernaemontana alfari Donn. Sm.	Costa Rica	
Tabernaemontana allenii (Woodson) A.O. Simões & M.E. Endress	Panama	*C. Goldames & N. Gomez 353* (INB, PMA)
Tabernaemontana eubracteata (Woodson) A.O. Simões & M.E. Endress	El Salvador	*M. Renderas 6/1997* (INB)
Tabernaemontana tomentosa (Greenm.) A.O. Simões & M.E. Endress	México	
Tabernaemontana sp nova	Costa Rica	
Tabernaemontana longipes Donn. Sm.	Costa Rica	
Tabernaemontana albiflora Rojas Acosta	Guyana	*M.J. Jansen-Jacobs et al. 5626*
Tabernaemontana lagenaria Leeuwenb.	Peru	*A.P. Macedo 1038* (INB)
Tabernaemontana panamensis (Markgr., Boiteau & L. Allorge) Leeuwenb.	Equador, Esmeraldas.	*E. Nováes et al. 1081* (QCNE, MO, INB)
Tabernaemontana stenoloba Müll. Arg.	Colombia	*R. Fonnega et al. 6396* (HUA, INB)

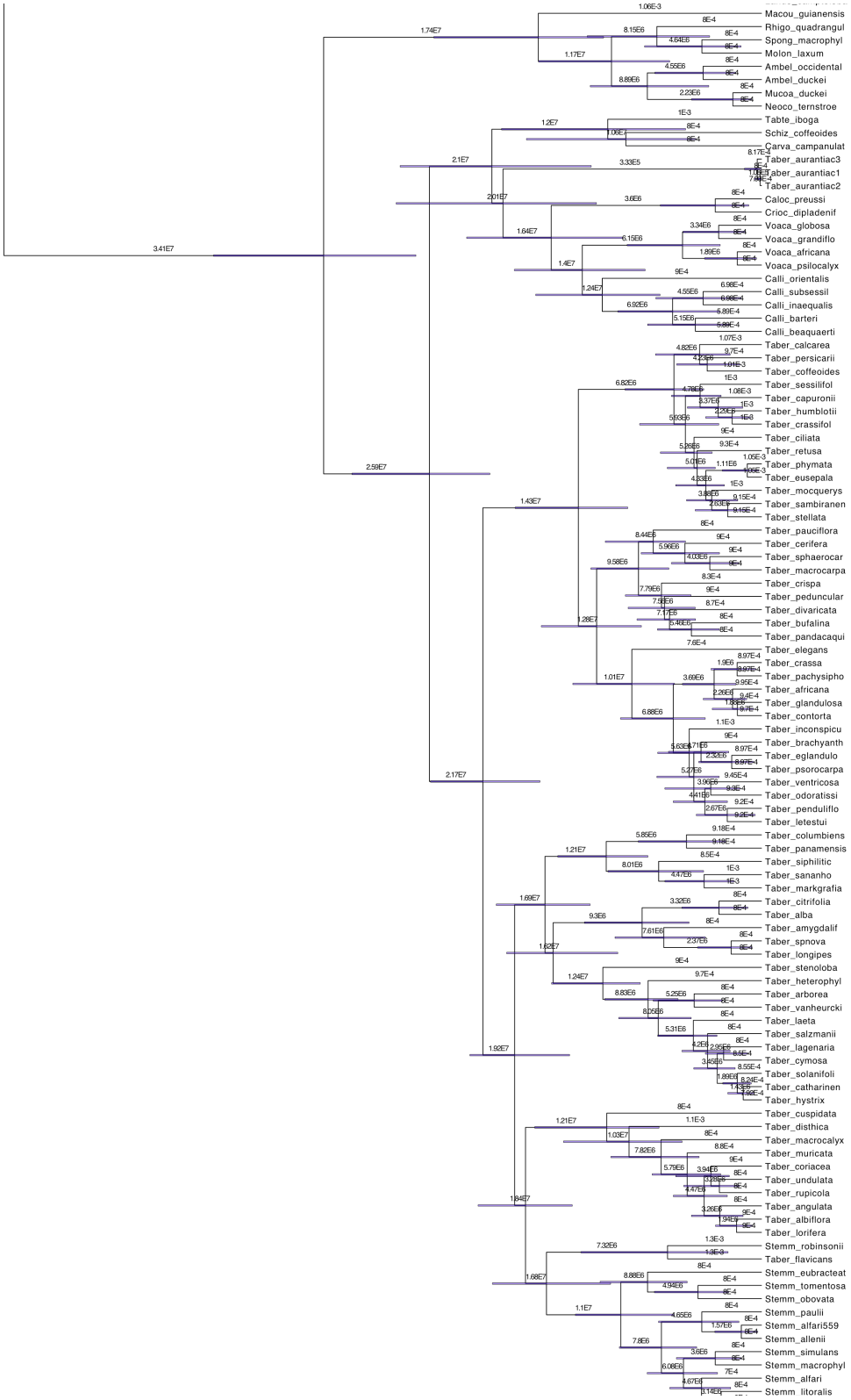
Appendix 2: A maximum clade credibility chronogram of ViWiTa clade based on plastid sequence (rps16, rpl16, trnS-G, trnK and matK) as inferred in BEAST obtained under a relaxed clock. Median ages are given above branches into scientific notation and node bars indicate 95% HPD (Highest Posterior Density) age ranges.



Appendix 2: continued



Appendix 2: continued.



CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

O estudo filogenético molecular de Willughbeieae (Capítulo 1) confirmou a monofilia da tribo. Os clados formados correspondem as subrtribos propostas atualmente por Endress *et al.* (2014). O gênero africano *Landolphia* é parafilético e para obtermos uma classificação monofilética os gêneros *Ancylobothris, Chamaeclitandra, Clitandra, Cylindropsis, Dyctiophleba, Orthopichonia, Pacouria, Saba* e *Vahadenia* foram sinonimizados em *Landolphia s.l*. A tribo atualmente compreende nove gêneros: *Bousigonia*, *Couma*, *Cyclocotyla*, *Hancornia*, *Lacmellea*, *Landolphia s.l.*, *Leuconotis*, *Parahancornia* and *Willughbeia*. Seis sinapomorfias foram sugeridas para a tribo: (1) hábito liana; (2) ápice da cabeça do estilete indiferenciado; (3) base da cabeça do estilete indiferenciado; (4) ovário sincárpico; (5) placentação parietal; e (6) fruto baga.

A partir da hipótese filogenética gerada, outros estudos podem ser realizados para elucidar as relações infragenéricas dos nove gêneros da tribo, como *Landolphia s.l.* o gênero africano mais diverso da tribo com cerca de 60 spp., ou o pequeno genero neotropical com cinco espécies, *Couma,* distribuído principalmente na Floresta Amazônica e com uma espécie com uma interessante disjunção entre os tepuis na Venezuela e a Chapada Diamantina na Bahia.

O estudo filogenético de Willughbeieae também veio a completar a filogenia do clado ViWiTa (Vinceae, Willughbeieae, Tabernaemontaneae) e propiciou a realização da datação molecular do clado (Capítulo 2).

Com a datação molecular e a reconstrução de area ancestral foi possível inferir uma provável origem do clado no inicío do Paleoceno na Australásia. A similaridade de padrões de disjunção entre Neotrópicos e Australásia observada nas três tribos do clado ViWiTa aconteceram quase ao mesmo tempo no Oligoceno e isso leva a acreditar que os processos envolvidos foram os mesmos. Essa cladogênese pode ser explicada pela ruptura da flora Boreotropical existente durante o Paleoceno-Eoceno na Laurásia e também por vários eventos de dispersão à longa distância.

Diversos estudos poderão ser realizados com a filogênia bem amostrada e datada de ViWiTa, como por exemplo evolução de estruturas morfológicas, inovações chave, análises de diversificação, e estudos biogeográficos na região Neotropical.

Seria interessante também tentar amostrar as espécies de alguns gêneros que foram pouco amostrados, principalmente os asiáticos, que possuem uma porcentagem menor no número de espécies amostradas. Isso seria de grande importância para entender os fatores bióticos ou abióticos envolvidos nas taxas de especiação e extinção nas diferentes regiões tropicais da terra.